CHASE:
Cardiovascular health in mothers and offspring after pregnancies complicated by preeclampsia and diabetes mellitus.

Focus on diet and physical activity

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Master of science thesis in clinical nutrition

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Preface

This master thesis presents preliminary results obtained from the first and clinical part of a still ongoing research project at Oslo University Hospital (OUH), Ulleval; "CHASE; Cardiovascular health in mothers and offspring after pregnancy complications." The work of this thesis has been conducted between August 2008 and May 2009 under the main supervision of Associate Professor and project leader Anne Cathrine (Annetine) Staff at the Women's Clinic OUH, Ulleval, and with co-supervision of Professor Lene Frost Andersen at the Department of Nutrition, University of Oslo.

First and foremost I would like to express my gratitude to Annetine and Lene, who both have contributed with valuable help and guidance during the year.

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Anne Stine Kvehaugen

Abstract

Background: Epidemiological evidence suggests an increased risk of cardiovascular associated diseases (CVAD) in both mother and offspring after pregnancies complicated by preeclampsia (PE) and diabetes mellitus (DM). The mechanisms behind this altered risk in health status are still scarcely unravelled. However, PE, DM and cardiovascular diseases (CVD) have several features in common, including shared risk factors and similar pathophysiology. Components of the metabolic syndrome and endothelial dysfunction, assumed to be closely related to nutritional intake and physical activity level, have gained increased attention as unifying mechanisms. Foetal programming in utero, as well as birth weight and growth pattern in the early postnatal period, may also predict future disease risk. The knowledge regarding the relative contribution from ”intrauterine programming” and nutritional and metabolic risk factors for the development of cardiovascular health and disease is limited, and is a subject for further research.

Objectives: In the present study we wanted to explore if there are any differences in lifestyle factors 5-8 years after delivery between women previously diagnosed with PE or DM in pregnancy and women without these complications, and likewise between their offspring. The lifestyle factors included in the master thesis were nutrition and physical activity. Also, associated risk factors for CVAD, including anthropometrics, blood pressure (BP), glucose levels and non-invasive measurements of endothelial function were evaluated.

Subjects: Women previously recruited to a caesarean section biobank in 2001-2004, 18 of which where diagnosed with PE, 14 of which had DM (8 gestational diabetes mellitus (GDM) and 6 DM1) and 9 subjects with uncomplicated pregnancies serving as a comparator group, agreed to participate in the present follow-up study, together with the child that was delivered and recruited to the original study in 2001-2004.

Methods: Height, weight, waist and hip circumferences, BP, endothelial function (non-invasive technique; EndoPAT) were measured in both mother and child. Both

also delivered a urine sample (which was analysed with a urine stix to detect glucosuria or proteinuria), and venous blood samples were collected (for future analysis in other planned sub studies). An oral glucose challenge test (OGT) was performed in the mother, with one reading in the fasting state at baseline and one reading 2 hours later after oral intake of 75 mg glucose in 300 ml water. Additional clinical information, as well as information regarding nutritional intake and physical activity level in both mother and child, were obtained from standardized questionnaires. The children also had a cardiac tissue Doppler ultrasound examination performed by a paediatrician, but the findings obtained from the Doppler study and the endothelial function assessment are not reported in the present master thesis (as the analyses will take another 6 months and exceed the scope of this master thesis).

Main outcomes/findings: In the CHASE follow-up study, a higher proportion of women and children in the DM-group were overweight and obese compared to the other groups. More women in the DM- and PE-group had abdominal obesity according to waist-to-hip ratio (WHR) measurements compared to the C-group. We also detected significant higher systolic BP and a lower physical activity level in women with previous PE or DM. Low intakes of vegetables were identified in all groups. However, a larger proportion of children in the C-group followed the fruit dietary guidelines of two or more portions a day compared to the DM-group ($p = 0.02$) and the PE-group ($p = 0.08$) of children. Also, a non-significant larger proportion of the women in the C-group reported adhering to the fruit dietary guidelines compared to the two other groups of women. Women and children in the C-group had a higher consumption of cod liver oil and lean milk types as compared to the other study groups.

Conclusion: Preliminary results of the CHASE clinical study have identified aims for potential intervention strategies in women and children after pregnancies complicated by PE, GDM or DM1. Such intervention could focus on maternal weight reduction and prevention of excessive weight gain in the children, increase in physical activity level among the women, and advice to increase the consumption of fruit/berries and

vegetables, less sugar-sweetened beverages, promotion of lean milk types rather than higher fat milk types and possibly the use of cod liver oil supplements.

Key words: Preeclampsia (PE), diabetes mellitus (DM), gestational diabetes mellitus (GDM), cardiovascular disease (CVD), Cardiovascular associated diseases (CVAD) metabolic syndrome, endothelial dysfunction, foetal programming, nutrition, physical activity.

Abbreviations

AGE: Advanced glycated end products

AHA: American Heart Association

BMI: Body mass index

BP: Blood pressure

C: Control

CHD: Coronary heart disease

CVAD: Cardiovascular associated disease

CVD: Cardiovascular disease

Diastolic BP: Diastolic Blood pressure

DM: Diabetes mellitus

DM1: Diabetes mellitus type 1

DM2: Diabetes mellitus type 2

FFQ: Food frequency questionnaires

DD: Diet diaries

GA: Gestational age

GDM: Gestational diabetes mellitus

HDL: High-density lipoprotein

Index pregnancy: in the CHASE study: defined as the pregnancy ending with a delivery in 2001-4, where the participants in the present CHASE study (2008-9) were recruited for the first time to the biobank study.

IUGR: Intrauterine growth restriction

LDL: Low-density lipoprotein

MUFA: Monounsaturated fatty acids

n-3 PUFAs: Omega-3 polyunsaturated fatty acids

n-6 PUFAs: Omega-6 polyunsaturated fatty acids

NO: Nitrogen Oxide

OGT: Oral glucose challenge test

OUH: Oslo University Hospital

PE: Preeclampsia

ROS: Reactive oxygen species

BP: Blood pressure

TAG: Triacylglycerol/ triglycerides

WFR: Weighed food records

WHR: Waist/hip-ratio

WHtR: Waist/height-ratio

24HR: 24 hour recall

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1. Introduction

1.1 Cardiovascular disease in women and the significance of pregnancy complications

Accounting for one third of all deaths, cardiovascular disease (CVD), which include coronary heart disease (CHD), stroke and peripheral vascular disease, is the largest single cause of death among women worldwide. In many countries, the mortality rates from CVD in women even exceed those of the men's.

Most CVD in both women and men is preventable, and modifiable risk factors, some of which are also interconnected, include cigarette smoking, poor diet, physical inactivity, obesity, hypertension, dyslipidemia, metabolic syndrome and diabetes mellitus type 2 (DM 2) (22).

A risk factor unique to women, is the increased likelihood of developing CVD after pregnancy complications such as preeclampsia (PE) and other placental syndromes. Whether this reflects a cause and effect relationship, or rather is due to an underlying condition that predisposes women to both conditions, is uncertain. An analogous situation is the increased risk of developing diabetes mellitus type 2 (DM2) after a history of gestational diabetes mellitus (GDM).

In either case, pregnancy represents an opportunity to identify women at increased CVD risk. Given the prevalence of CVD in women, targeting these individuals for primary prevention postpartum could be very valuable from a public health perspective. As suggested by Newstead et. al, education about healthy lifestyle choices could have a positive impact not only on the woman herself, but also on the rest of the family members (22, 23, 24).

1.2 Preeclampsia

1.2.1 Clinical picture

Preeclampsia is a multisystem vascular-related disorder of pregnancy originating in the placenta. It is characterized by the occurrence of hypertension (≥140/90 mmHg) and proteinuria (≥ 0.3 g/24 h) after 20 weeks of gestation, in a previously normotensive woman (1).

It has been suggested to subdivide the condition into early-onset (< 34 or 37 weeks gestation) and late-onset (> 34 or 37 weeks gestation) disease (2).

About 2-7 % of healthy nulliparous women are affected by preeclampsia (3), with approximately 93% of cases developing at 34 weeks gestation or later (4). In Norway, the Medical Birth Registry of Norway reported an 3.7% incidence of preeclampsia in 2004 (89). Most preeclampsia patients are delivered close to term, 2/3 with delivery at gestational age (GA) ≥ 37 weeks, and 1/3 with a GA <37 weeks.

Preeclampsia may further progress to the more severe forms, eclampsia (seizures resulting from cerebral edema or cerebral hemorrhage, often with headache and visual changes as initial symptoms) and HELLP (hemolysis, elevated liver enzyme levels and a low platelet count) syndrome (19-20).

The offspring can also be adversely affected by the condition. Intrauterine growth restriction, preterm birth (most often iatrogen) and foetal death representing the most severe complications. (1).

According to the World Health Organisation (WHO), the hypertensive disorders of pregnancy, preeclampsia and eclampsia, collectively accounts for 12 % of maternal deaths worldwide (18). Preeclampsia is responsible for approximately 50 000 maternal deaths annually (90).

1.2.2 Long-term implications for both mother and offspring

Several epidemiological studies have detected an association between a history of PE and an elevated risk of future hypertension, stroke, venous thromboembolism, ischaemic heart disease and death from any cause.

The risk seems to be even higher with more severe manifestations of PE, such as severe hypertension (systolic BP ≥ 160 mm/Hg and/or diastolic BP ≥ 110 mm/Hg) associated with proteinuria, or hypertension associated with severe proteinuria (≥ 5g/day), multiorgan involvement, PE associated with intrauterine growth restriction (IUGR), pre-term birth and foetal death, early onset PE (< 37 weeks gestation) and recurrent PE (24-25, 72).

Offspring of preeclamptic pregnancies might also be at increased risk for future CVAD, and several studies have reported higher diastolic and systolic BP in children and adolescents subjected to a preeclamptic pregnancy compared to control subjects (80-83). However, after adjustment for BMI in one of these studies, and adjustment for birth weight, current weight and BMI in another, the observed differences attenuated (82-83).

1.3 Diabetes in pregnancy

1.3.1 Gestational Diabetes Mellitus

GDM is defined as glucose intolerance with onset or first recognition during pregnancy (26), and women with glucose levels in the top 5-10 % of the population distribution meet the diagnostic criteria (27). The condition is associated with insulin resistance as well as impaired insulin secretion (68).

GDM occurs in about 4 % of pregnancies and accounts for nearly 90 % of diabetic pregnancies. Women diagnosed in the first half of pregnancy represent a high-risk sub group, with an increased risk of obstetric complications, recurrent GDM in subsequent pregnancies, and postpartum development of DM2.

Also, gestational hypertension, PE and essential hypertension seems to be more frequent in women with GDM, an association suggested to be linked with insulin resistance (26, 68, 71).

1.3.2 Long-term implications for both mother and offspring

It is well established that women with a history of GDM are at increased risk for subsequent development of DM2. The high prevalence of cardiovascular risk factors in these women, such as obesity, insulin resistance, chronic hypertension and metabolic syndrome, theoretically place them at increased risk of future development of CVD as well (77, 79).

Intrauterine exposure to diabetes can result in excess foetal growth and macrosomia, due to increased maternal glucose transfer across the placenta. Because maternal insulin does not cross the placenta, foetal insulin, which itself acts as a growth hormone, is produced in elevated amounts. A review including two prospective studies that have examined the role of intrauterine exposure to diabetes on childhood growth and later risk for obesity and DM2; the Pima Indian Study and the Diabetes in Pregnancy Study, supports the evidence that offspring of diabetic pregnancies, regardless of maternal diabetes type, are at high risk for obesity and DM2 later in life (84).

A cohort study demonstrated that higher birth weight, and having been born to a mother with GDM, both independently predicted increased risk of overweight in adolescence. After adjustment for the mother's own BMI, the association with GDM attenuated (85).

1.4 What is the association between cardiovascular disease, preeclampsia and diabetes mellitus?

1.4.1 Shared risk factors and similar pathophysiology

Insulin resistance and the metabolic syndrome

The metabolic syndrome, which is a spectrum of metabolic abnormalities that are related to overweight and insulin resistance, is a key factor underlying atherosclerotic CVD and DM2 (30). As defined by The National Cholesterol Education Program Expert Panel on Detection, Evaluation, and treatment of High Blood cholesterol in Adults (ATP-III) criteria, the metabolic syndrome is characterized by the presence of three or more of the following; waist circumference > 102 cm in men and 88 cm in women; serum triglycerides (TAG) level ≥ 150 mg/dL (1.69 mmol/L); HDL (High-density lipoprotein) cholesterol level < 40 mg/dL (1.04 mmol/L) in men and 50 mg/dL (1.29 mmol/L) in women; BP ≥ 130/85 mmHg; or serum glucose level ≥110 mg/dL (6.1 mmol/L) (29, 145). Additional components recently recognized, but currently recommended only for research purposes, include proinflammatory and prothrombotic markers (30).

During pregnancy, physiological changes occur that results in a transient metabolic syndrome characterized by insulin resistance, hyperlipidaemia, up-regulation of the inflammatory cascade and an increase in white cell count and coagulation factors. Pregnancy complications such as PE and GDM can be regarded as a failure to "the pregnancy stress test", in which there is an exaggerated response to these metabolic and vascular changes that normally occurs during pregnancy (31).

A large retrospective cohort study published in 2005 investigated the association between features of the metabolic syndrome and placental dysfunction (including, but not limited to PE) in 1.03 million Canadian women with a documented first delivery. Any features of the metabolic syndrome were characterized up to 24 months before their index delivery. The results indicated that women with pre-pregnancy features of the metabolic syndrome had a higher graded risk of placental dysfunction and foetal

death (69). A population-based study of 3494 Norwegian women, published in 2007, found positive associations between serum levels of TAGs, LDL cholesterol and non-HDL cholesterol, as well as BP, obesity and waist circumference measured before pregnancy, and subsequent risk of PE. These results indicate that risk factors for CVD, which are also components of the metabolic syndrome, are evident years before the clinical presence of PE (70). Also, a systematic review of 13 cohort studies, comprising nearly 1.4 million women, published in 2003, found a strong positive association between pre-pregnancy BMI and the risk of PE (73).

Pre-pregnancy BMI is also associated with the development of GDM, recurrent GDM and subsequent development of DM2 (68, 76-77).

Evidence also indicates that features of the metabolic syndrome can persist postpartum in women with previous hypertensive pregnancy (74-75) and GDM (78-79).

Endothelial dysfunction

Physiological function of the endothelium

The endothelium is the cell-layer lining the internal surface of blood vessels, and serves as an interface between the vessel wall and circulating blood. It plays a substantial role in regulating vascular homeostasis, tone and structure (33-34).

Definition

Endothelial dysfunction is defined as "an alteration of the regular function of the endothelium that leads to prothrombotic, pro-inflammatory, proproliferative, and proconstrictive activation of the vasculature and is the consequence of insufficient repair to removal or functional injury of endothelial cells" (33).

Endothelial dysfunction in atherosclerosis

Several hypotheses regarding the phatophysiology of atherosclerosis have been proposed, including "the response to retention hypothesis" which suggests lipoprotein-matrix interactions as the inciting event in atherosclerosis, "the oxidative

modification hypothesis" that focuses on the oxidation of LDL lipids as the critical event in atherosclerosis delelopmenet, and "the response to injury hypothesis".

According to "the response to injury hypothesis", endothelial dysfunction is recognized as the initial event in atherogenesis, resulting from an injury to the vascular wall. This disturbs the balance between vasoconstriction and vasodilation and initiates events that are involved in the development of atherosclerosis, such as increased endothelial permeability, enhanced adhesiveness of the endothelium to leukocytes and platelets and the formation of cytokines (34-35, 91).

Endothelial dysfunction in preeclampsia

The patophysiology of PE is not completely understood, but defective placentation seems to be an essential event.

Normal placentation requires that extravillous cytotrophoblasts, a specialized population of the outer cell mass of the blastocyst, invade the maternal spiral arteries of the uterine wall. During the remodelling of the spiral arteries into large-capacity, low-resistance arteries, the extravillous cytotrophoblasts invade the inner part of the endometrium (called decidua in pregnancy) and myometrium and replace the smooth muscle layer in the vessel wall with a fibrinoid layer. Also, the trophoblast temporarily replaces the endothelial lining (92).

In women who develop preeclampsia, there is a failure of the cytotrophoblasts to differentiateinto an endothelial phenotype and the invasion of the myometrium will remain shallow. Consequently this will lead to a defective spiral artery remodelling with a lack of "physiological change". The spiral arteries will remain narrow, tortuous and thick-walled, with intact smooth muscle cell layer. This non-transformation of spiral arteries in preeclampsia results in an altered uteroplacental circulation and subsequent placental oxidative stress and possibly ischemia, as the demands of the growing fetoplacental unit increases (19, 20,21). It is widely believed that an oxidatively stressed and possibly ischemic placenta will release prothrombotic, proinflammatory, vasoconstrictive and antiangiogenic substances (24), which can induce the endothelial dysfunction of the maternal vascular system.

There are also findings of increased lipid peroxidation and oxidative stress in the placentas of preeclamptic women (141-143). This supports the theory that abnormal placentation combined with dyslipidemia can result in the release of free radicals and lipid hydroperoxides and thus oxidative stress, believed to cause endothelial damage both locally and in the systemic circulation (23, 32).

In placentas of preeclamptic women, there are often areas of lipid deposition in the non-transformed spiral artery walls, called acute atherosis, owing to its similarity to the early stages of atherosclerotic lesion with lipid deposition (24).

Endothelial dysfunction in diabetes mellitus

Increasing evidence suggest that endothelial dysfunction is a contributor to the pathogenesis of vascular disease in DM.

It has also been proposed that there could be a close link between endothelial dysfunction and insulin resistance, as the progression of insulin resistance to DM2 seems to accompany the development of endothelial dysfunction to atherosclerosis. Impaired endothelial function in large arteries is central to the pathogenesis of CVD, while endothelial dysfunction in the capillaries and arterioles is a likely key step in the development of insulin resistance. (36-38).

In established diabetes, hyperglycemia plays a significant role in the development of endothelial dysfunction and progression of chronic diabetic complications. One of the possible pathways involved include the formation of irreversible advanced glycated end products (AGE). Adverse effects of AGE to the vasculature includes changes in extracellular matrix components, generation of ROS, increased expression of inflammatory mediators, growth factors and adhesion molecules. AGE also promote the oxidation of LDL. In addition to glycated lipids, oxidized LDL are easily taken up by macrophage scavenger receptors, with the subsequent formation of foam cells and atherosclerotic plaques (38-40). Circulating AGE was found elevated in pregnancies complicated by DM, but not in PE in a previous study from our biobank cohort; suggesting a biological heterogeneity (138)

1.4.2 Developmental origins of health and disease

The susceptibility to many late onset chronic diseases has been proposed to be related to events in early life. This assumption rests mainly on evidence obtained from experimental animal models (7), as well as from epidemiological studies linking low birth weight or disproportional foetal growth with later onset CVAD, including non-insulin dependent DM (5-6). This has led to the hypothesis of "foetal origins" and "foetal programming", which proposes that the foetus make adaptations in response to the intrauterine nutritional, metabolic and hormonal milieu. These effects will be beneficial for immediate survival. However, if obtained during critical periods of development, when developmental plasticity is most pronounced, the effects will also persist after birth. In this context, IUGR might result as a consequence of placental insufficiency in PE, while macrosomia and congenital malformations might result as a consequence of maternal diabetes. In other terms, the foetus has been programmed to survive under conditions similar to what was experienced in utero. If a mismatch occur between the intra- and the extra-uterine conditions, the resultant phenotype may therefore be unfavourable in the long term health perspective (8-11, 86).

Recent evidence suggest that epigenetic processes (defined as changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence) might be involved in the foetal adaptations in utero, with the consequence that these effects also can be passed on to successive generations (12, 13).

In addition to size at birth, the early postnatal growth pattern also seems to play a role for health in later life. As reviewed by Barker, slow growth in foetal life and infancy, combined with a rapid increase in BMI after the age of 2 years, is associated with the development of CHD and DM2 (14). Other recent publications links the risk of becoming obese with certain patterns of growth and weight gain in the first years of life. Weight gain during the first 6 months of life (15, 16), and from 2 years onwards (16), seems to correlate with later risk of obesity. In a review by Huxley et al., evidence also indicates that postnatal catch-up growth is positively associated with BP in adult life (17).

1.5 Influence of nutrition and physical activity on cardiovascular disease and associated conditions

1.5.1 The metabolic syndrome and its components

Overweight and obesity

The metabolic syndrome typically occurs in the setting of overweight or obesity, and there seems to be general agreement in the literature that weight reduction should be one of the primary approaches in the treatment of this condition (47, 48-50). This can be achieved by increasing the physical activity level and by reducing the energy content of the habitual diet (49-50, 63-64).

Insulin resistance and plasma glucose

Insulin sensitivity often deteriorates in overweight or obese individuals, particularly in the presence of visceral adiposity. Thus, loss of body weight can improve insulin sensitivity and also exert beneficial effects on the other abnormalities in the metabolic syndrome, even if the ideal body weight is not achieved (48-49).

The type and amount of fat also seems to play a role, with saturated and trans-fatty acids reducing insulin action, and unsaturated fat, with strong evidence for monounsaturated fat, improving the action of insulin (48-49, 51-53). However, these effects are abolished when total fat intake increases from 20 to 40% (48).

Foods with a high glycemic load, that is; easily absorbed carbohydrates, may aggravate glucose intolerance and dyslipidemia. Individuals who are obese and have insulin resistance are probably more prone to the metabolic effects of a high dietary glycemic load (54).

Plasma lipid profile

Type and amount of dietary fat also influences the levels and distribution of plasma lipids. Saturated fat is strongly and positively associated with plasma LDL-cholesterol levels and may also increase BP. Dietary cholesterol is also associated

with elevated levels of LDL-cholesterol. n-3 PUFAs reduce serum levels of TAG and may lower BP in hypertensive individuals. n-6 PUFAs reduce LDL-cholesterol and TAG levels but also reduce HDL-cholesterol levels, while MUFAs reduce LDL-cholesterol and increase HDL-cholesterol levels, and may exert a reductive effect on BP (53).

Blood pressure

Although dietary fat may exert some effects on BP, evidence strongly supports that a dietary pattern in line with the DASH (Dietary Approaches to Stop Hypertension) diet has a pronounced effect in lowering BP. This diet emphasizes a high intake of fruits, vegetables and low-fat dairy products and a reduced intake of saturated- and total fat and cholesterol. The diet also includes whole grains, nuts, fish and poultry, while the inclusion of red meat, sweets and sugar-containing beverages are sparse. Especially important is the low salt (NaCl) content of this diet, as salt is an independent predictor of elevated BP (55). Weight reduction and moderation of alcohol consumption among those who drink alcohol can also lower BP (47).

1.5.2 Endothelial dysfunction

In a 2001 review on the topic “Dietary modulation of endothelial function: implications for cardiovascular disease”, several dietary factors are highlighted as potential modulators. These include: n-3 PUFAs, in part by their in vitro anti-inflammatory effects and their ability to increase the production of nitric oxide (NO), which is an endothelium-derived vasodilator, and to improve hemostatic factors in vivo; antioxidant vitamins, with their potential to reduce oxidative stress within the body; folic acid, due to its ability to reduce plasma concentrations of homocysteine, again related to increased expression of adhesion molecules, platelet aggregation and decreased NO-production; and L-arginine, which is the substrate for NO-synthase that catalyses the formation of NO (42).

Although promising, results regarding single nutrients are conflicting and currently there are no specific recommendations on the amount of micronutrients, L-arginine or

n-3 fatty acids to be consumed, neither in the diet, nor as supplements, for the primary prevention of CVD.

The dietary pattern rather than single nutrients was the focus of a sub-trial based on the Nurses` Health study cohort, investigating the effect of diet-quality scores on plasma concentrations of markers of inflammation and endothelial dysfunction. Two dietary patterns were identified to be strongly associated with lower concentrations of biomarkers associated with adverse health effects. The dietary patterns identified were both characterized by a high consumption of fruits, vegetables, legumes, nuts, whole grains or cereal fiber, less red meat and saturated fat, and a moderate alcohol consumption. The main difference between these two diet-quality scores was the inclusion of fish in one, and the inclusion of long-term multivitamin use in the other. (43).

Lifestyle interventions, such as dietary modification and physical activity, in adults as well as adolescents and children, have also shown to be successful in improving arterial function (44-46). The latter finding may be especially important, due to the fact that the atherosclerotic process, manifested as so-called fatty streaks, can be evident already in early childhood (44).

1.5.3 Cardiovascular disease

A review published in 2002 by Hu and Willett, summarizing 147 original articles and reviews of epidemiologic studies, metabolic studies and dietary intervention trials of diet and CHD, concludes that there is substantial evidence indicating that a high consumption of fruit, vegetables and whole grains, using non-hydrogenated unsaturated fats as the preferentially source of fats as well as adequate intake of n-3 PUFAs can offer significant protection against CHD. The review also suggests that such dietary patterns in combination with physical activity, avoidance of smoking, and maintenance of a body weight in the normal range, may prevent the majority of CVD in western populations (56). Among the studies to support this issue, are the findings of an inverse association between fruit and vegetable consumption and CVD

(57-59), whole grain consumption and ischemic stroke in women (60), fish and n-3 PUFA consumption and ischemic stroke (61-62), physical activity and CVD events in women (65,66-67).

2. The CHASE study

2.1 Background

The CHASE study is a follow-up study of women and offspring after pregnancies complicated by preeclampsia and/or diabetes mellitus, combining clinical and molecular findings at delivery with clinical and molecular findings, as well as information about nutritional intake and physical activity level, 5-8 years later.

To our knowledge, few if any publications to date have investigated the nutritional intake and physical activity level in mothers and offspring after pregnancies complicated by diabetes mellitus or preeclampsia.

Bearing in mind the epidemiological evidence for an increased risk of CVAD, including DM and endothelial dysfunction, in both mother and offspring later in life, an antiatherogenic lifestyle, avoiding excessive calories and physical inactivity, would presumably be of major health benefit in this at risk population.

2.2 Objective

The aim of the CHASE study is to further explore some of the potential mechanisms responsible for future development of CVAD, including DM and endothelial dysfunction, in both women and children after pregnancies complicated by PE or DM, enabling us to later possibly offer specific intervention strategies to optimalize health in this population subgroup.

The master thesis will mainly focus on dietary and physical activity data obtained during the first and clinical part of this still ongoing research project.

2.3 Hypothesis

One of our hypotheses was that an “atherogenic” or “obesogenic” lifestyle at least partly contribute to the epidemiologically demonstrated increased risk for CVAD later in life, in both women and offspring, after PE or DM in pregnancy.

Based on this assumption, we wanted to investigate whether there are any differences between women and offspring of preeclamptic versus diabetic pregnancies, or between any of these two groups compared to women and offspring of uneventful pregnancies, in any of the following variables:

1. Anthropometrics

2. Blood pressure

3. serum glucose levels

4. Dietary intake

5. Physical activity

Specifically;

1. Do women and/or children 5-8 years after pregnancies complicated by PE and/or DM have a higher BMI, waist circumference, waist/hip ratio and waist/height ratio (children) compared to women and/or children who did not have these complications 5-8 years ago?

2. Do women and/or children 5-8 years after pregnancies complicated by PE and/or DM have a higher BP compared to women and/or children who did not have these complications 5-8 years ago?

3. Do women 5-8 years after pregnancies complicated by PE and/or GDM have a persistent glucose intolerance/ hyperglycemia, and are there any differences between any of these groups and the control group regarding glucose levels in the fasting state and/or 2 hours after an oral load of 75 g of glucose?

4. Do women and/or children 5-8 years after pregnancies complicated by PE and/or DM have a more unfavourable dietary intake compared to women and/or children who did not have these complications 5-8 years ago?

5. Do women and/or children 5-8 years after pregnancies complicated by PE and/or DM have a more sedentary lifestyle compared to women and/or children who did not have these complications 5-8 years ago?

2.4 Subjects

The clinical basis of the study are 156 women previously recruited to a biobank study at Oslo University Hospital (OUH), Ulleval in Oslo, during 2001-2004, due to pregnancy complications such as PE (42 women), GDM (39 women), pre-GDM (29 women, of which 23 women with DMI and 6 with DMII), as well as women with uneventful pregnancies (46 women). Most preeclamptic pregnancies were delivered prematurely, in contrast to the diabetic and uneventful pregnancies, being delivered mostly at term. All women were recruited to the biobank project at time of delivery, or close to delivery, and agreed at that time, by signed informed consent, to be contacted and asked to participate in potential follow-up studies.

In the present CHASE study we aimed at recruiting the same mothers and their offspring from this pregnancy biobank, 5-8 years after the index delivery.

Exclusion criteria: current pregnancy, current lactation.

2.5 Study approvals

Inclusion in the CHASE study is based on a new voluntary signed informed consent from the woman on behalf of herself and her child. The women were explicitly informed that they could refuse to participate or later withdraw from the study at any time without giving any reason and without any consequences regarding the relationship to OUH.

The original biobank study is approved by the Regional Committee for Medical and Health Research Ethics, Eastern Norway (REK Øst), permission from the Norwegian Data Inspectorate (Datatilsynet) has been obtained, and the biobank has been approved by the Ministry of Health and Care Services (Helse- og omsorgsdepartementet).

The present CHASE study is approved by the Regional Committee for Medical and Health Research Ethics Southern-Eastern Norway (REK Sør-Øst) as well as by the Data Inspectorate (konsesjon). Prior to REK evaluation, all formal approvals internally at OUH, Ulleval, were provided, including local (Head of Department of Obstetrics and Gynaecology) and divisional (Women and Children Division, head of research committee) approval, data inspectorate officer (personvernombud), biobank officer and formal registration and approval of the project at OUH, Ulleval. The CHASE biobank is formally approved by the directorate of Health (Helsedirektoratet).

2.6 Study design

An invitation to participate was sent to all 156 women from August 2008 (appendix 1). Those who did not reply after a few weeks, were sent a reminder and/or tried to be contacted by phone.

All women who agreed to participate received two short questionnaires about physical activity and nutrition, one for herself and one for her child, which she completed before the clinical visit at the hospital.

The clinical visit lasted for approximately 2-3 hours and included breakfast and time to relax and play for the child.

The woman and her child arrived the hospital after an overnight fast, at 8 am. Both delivered a urine sample for urine sticks analysis, and venous blood samples were collected for future analysis of routine haematology and potential biomarkers. In the

case of the mother, an oral glucose challenge test was also undertaken, with a second blood sample 2 hours later for the measurement of serum glucose level.

A tissue Doppler cardiac analysis was performed in the child by an experienced cardiologist paediatrician.

BP (repeated 3 times after resting), and anthropometrics such as height, weight, waist and hip circumferences, were measured in both mother and child, as well as a non-invasive endothelial function examination, using the "EndoPAT 2000" machine.

Additional clinical information was given by the mother, on the basis of a standardized questionnaire.

If the women were interested, they could receive feedback on clinical and/or dietary and physical activity data by the master degree student in nutrition.

2.7 Methods

2.7.1 Anthropometrics

Height

Height was measured in centimetres with one decimal, using an altimeter placed on the wall, with the participant standing straight, without shoes, and with feet gathered.

Weight

The participants were weighed without shoes and heavy clothes. Weight was measured in kilograms with one decimal, using a regular electronic scale.

BMI

Body mass index (BMI) was calculated according to the formula: weight (in kilo) divided by squared height (in meters): weight in kilograms/ (height in meters x height in meters).

Overweight and obesity in adults was defined as BMI ≥ 25 and BMI ≥ 30 respectively.

Iso-BMI in children was defined using the age- and sex-specific cut off points developed by Cole et al. (97).

Waist circumference

Waist circumference was measured to the nearest 0.5 cm, using a measuring tape of plastic.

Two different methods were used. In the first method, the waist circumference was measured at the narrowest level between the iliac crest and the lowest costa, while in the second method, the waist circumference was measured halfway between the iliac crest and the lowest costa. The data presented in this thesis on waist circumference are based on the latter method.

Hip circumference

Hip circumference was measured at the level of the great trochanters (not necessarily the widest circumference).

Waist to hip ratio

Waist to hip ratio (WHR) was calculated as waist circumference measured at the narrowest level between the iliac crest and the lowest costa, divided by hip circumference.

Abdominal obesity

Abdominal obesity in the women was defined as a waist circumference ≥ 88 cm according to the ATP-III criteria, or a WHR ≥ 0.85 according to the World health organization (WHO) cut-off points (138).

Abdominal adiposity (overweight and obesity) in the children was assessed based on age- and sex specific references for waist circumference in Dutch children developed by Fredriks et al. (132).

2.7.2 Blood pressure

BP was measured manually, with the BP cuff placed on the participant's dominant arm. Size of cuff (height) was chosen as approximately one third of upper-arm circumference. Three subsequent readings (with cuff in heart level position) were performed after the participant had rested for at least five minutes. Average systolic and diastolic BP was calculated as the mean of the two last readings.

2.7.3 Blood samples and OGT

Fasting blood samples were collected from both mother and child through an antecubital arm vein. All children were offered local anaesthesia of the skin prior to the test ("Emla plaster").

The mothers also performed a 2 hour OGT for detection of any abnormal glucose metabolism. According to the WHO (1999) criteria, the fasting serum glucose test was defined as elevated if ≥ 6.1 mmol/l and the OGT was defined as abnormal if the 2 h test was ≥ 7.8 mmol/l (138).

The OGT method is described in details in appendix 5.

Urine stix analysis

Both the women and the children delivered a urine sample during the hospital visit. The urine samples were analysed with a urine stix (Bayer™ reagent-stix for urine analysis) to detect any glucosuria or overt proteinuria

2.7.4 Clinical questionnaire/Clinical form

A clinical form was also developed to obtain additional information, such as number of pregnancies, number of births, menstruation, contraception, lactation, smoking, diseases and medications.

Data regarding blood volumes, urine sticks analysis, BP and anthropometrical measurements, were entered into this clinical form as well, by the student in nutrition.

The standardized clinical information sheet is presented in appendix 2.

2.7.5 Food frequency and physical activity questionnaires

In this study we have used two short pre-coded questionnaires about nutrition and physical activity, one for the participating mother, and one for the participating child respectively.

The mother received and filled out these two forms after she had agreed to participate on behalf of her self and her child. The completion of these forms, which each contain three pages, and eight (for the child) and eleven (for the mother) questions respectively, was estimated to take approximately 10-15 minutes.

The children's questionnaire is, with a few exceptions, identical to a food frequency- and physical activity questionnaire previous used and validated against a four-day precoded food diary in the Norwegian "UNGKOST 2000" study (87-88). A similar food frequency questionnaire (FFQ), used and validated against a 7-day precoded food diary in a health survey in Gjøvik, was the basis for the design of the women's questionnaire in the present CHASE study. A validation study on an earlier version of the questionnaire used in the health survey in Gjøvik has been published (135).

Both the women's and the children's questionnaire included questions about milk intake with different fat percentages, fruit juices, soft drinks with either sugar or artificial sweetening, bread with different fibre content, vegetables, fruits, berries, meat, fish, bread spreads, as well as the use of supplements, such as cod liver oil and

vitamins. Also a question about meal frequency was included. The main differences between the women's and the children's form, were that the women's questionnaire included questions about alcoholic beverages such as beer and wine, while questions about sweets, savoury snacks and fast foods such as pizza, hamburgers and hot dogs were included in the children's questionnaire. In both the women's and the children's questionnaire, frequency alternatives for beverages were; "never/seldom", "1-3 glass a month", "1-3 glass a week", "4-6 glass a week", "1-3 glass a day", "4-6 glass a day" and 7 glass or more a day", while frequency alternatives for the other food items were; "never/seldom", "1-3 times a month", "1-3 times a week", "4-6 times a week", "1 time a day", "2 times a day", "3 times a day" and 4 times or more a day".

Prior to statistical analysis, the frequency alternatives were recoded into "glass a day" and "times a day" for beverages and food items respectively. In the women's questionnaire, medium whole grain bread and whole grain bread were recoded into one group, while the same was done with sugar sweetened soda and lemonade, as well as artificial sweetened soda and lemonade, in the children's questionnaire.

The questions regarding physical activity among the women comprised how often, how long and with what kind of intensity physical activity was performed during the last seven days. Two questions about spare time physical activity were included, as well as a question about walking/bicycling to or from job/store etc. Frequency alternatives were single days. In the children's questionnaire the questions only comprised high-intensity physical activity, and only questions about spare time physical activity were included. These two questions were: "how often during a week-and how many hours during a week do you perform high intensity physical activity?" In contrast to the women's activity questionnaire, frequency alternatives were intervals, ranging from "never" to "every day" and "never" to "seven hours or more". There was also a question about sedentary behaviour, like watching TV or playing computer- or video games, with frequency alternatives ranging from "never" to "four or more hours a day".

Based on the women's questionnaire, an additional variable “total physical activity in hours a week” was computed for statistical analysis.

The combined physical activity and food frequency questionnaires for mother and child are presented in appendix 3 and 4 respectively.

2.7.6 EndoPAT 2000

EndoPAT 2000 is a non-invasive RH-PAT (Reactive Hyperemia Peripheral Arterial Tonometry) technique. The method is viewed as a reliable and sensitive measurement of endothelial function in both adults and children (129, 131-132).

With use of plethysmography, the PAT signal measures arterial pulsatile blood volume changes in the fingertip before and after 5 minutes occlusion of the brachial artery, and is a “window” to the arterial endothelial system and the autonomic nervous system (144).

The examination in the CHASE -study took place in a quiet room with dimmed light. Only the investigator (the master student or a paediatrician) and the participant were present. The participant was resting on a bed during the whole examination, which lasted for about 15 minutes.

Prior to the examination, BP was measured on the dominant arm (as previously mentioned under methods; Blood pressure).

Fingertip probes were then attached to the forefinger on each hand, and the finger on the non-occluded arm served as a control registration.

A blood pressure cuff was placed at the non-dominant arm and inflated to at least 200 mmHg for 5 minutes. Preceding the occlusion, baseline was measured for at least 5 minutes (5-6 minutes). Post-occlusion measurement also lasted for at least 5 minutes (5-6 minutes).

2.7.7 Statistical analysis

All statistics were performed using the Statistical Package for the Social Sciences (SPSS) version 14.0. Comparisons between the groups were analysed using the Mann-Whitney test, while the Wilcoxon signed rank test was used in paired analysis. Correlations were analysed using Spearman rank correlation coefficient. The level of statistical significance was set at $P < 0.05$. Data are presented as median values and values at the 25th and 75th percentile.

3. Results

3.1 The study population

Data from all women and children enrolled from the beginning of September 2008 to the end of February 2009 were included in the present master thesis. More participants have been and will be included after this time period, but are not included in the present thesis due to time limitation for the master thesis. During this period, 43 women consented to participate in the CHASE study; 9 of these women were categorized in the index pregnancy as belonging to the control group (C, uncomplicated pregnancies), 18 to the PE group and 16 to the DM group (8 with GDM and 6 with DM1). Two women, one with DM1 and one with GDM, were excluded due to current pregnancy, leaving a total number of 41 women and 43 children (24 girls (55.8 %) and 19 boys (44.2%)). All women in the C-group had a Norwegian ethnical background while 88.9 % and 75 % of the women in the PE- and DM-group respectively had a Norwegian ethnical background. There were no statistical significant differences between the study groups regarding ethnicity. Moreover, there were no statistical significant differences regarding distribution of higher educational level between the groups of women included. Nearly all of the women were non-smokers, and none of the women smoked on a regular basis (data not shown).

Median follow-up time from the index pregnancy to inclusion in CHASE clinical study was 7, 6 and 6.5 years for the C-, PE- and DM-group respectively.

3.2 Clinical characteristics of the women

3.2.1 Age

Median age in years at index delivery and in the CHASE clinical follow-up study was 33 and 39 in the C-group, 30.5 and 36 in the PE-group, and 32 and 39 in the DM-group. Differences between the study groups were not significant (table 1 and table 2).

3.2.2 Anthropometrics

Weight measured before or during early index pregnancy (table 1) and in the CHASE clinical study (table 2) did not differ between the groups of women. However, during this in-between-study period there was a significant higher weight gain of approximately 4.7 kg (median value) in the PE group and 3.7 kg in the DM group as compared to 0 kg in the C group (P=0.02 for both) (table 3).

Regarding maternal BMI in the index pregnancy (pre-pregnancy/early pregnancy BMI) and BMI at CHASE inclusion, there were no differences between the C-group and either the PE- or DM-group, but the DM-group differed significantly from the PE-group at both time points (table 1 and 2), with elevated BMIs. Similarly to weight, there was also a significant median increase in BMI in both the DM-group and the PE-group during the in-between-period, as compared to controls (table 3).

Waist and hip circumferences were measured in women at inclusion in CHASE clinical study. Although no significant differences were found in waist circumference between the groups in the CHASE clinical study, there was a significant difference in WHR between the C-group (median value: 0.84) and the DM-group (median value: 0.89) at this time point (table 2). This difference was also significant when comparing the C-group to each of the sub groups of the DM-group; DM1 and GDM (data not shown). BMI showed a significant correlation to both waist circumference and WHR in the women collectively at CHASE-inclusion ($P < 0.01$), with the strongest

correlation to waist circumference (r = 0.91 for BMI and waist circumference, and r = 0.54 for BMI and WHR respectively). Figure 1 depicts a scatterplot of the correlation between BMI and waist circumference in women for the 3 study groups.

3.2.3 Blood pressure

Systolic BP at inclusion in CHASE clinical study was significantly lower in the C-group (median value: 115 mmHg) compared to the PE-group (median value: 119.5 mmHg) and the DM-group (median value: 121.5 mmHg), while no differences existed for diastolic BP (table 2). These differences in systolic BP between the study groups were not present when measured before week 20 of the index pregnancy, given as mean systolic BP of first 20 weeks (table 1). As shown in table 3, there was a significant increase in systolic BP (median increase: 7 mmHg) and diastolic BP (median increase: 5 mmHg) from early pregnancy to time of CHASE inclusion in the PE-group, as well as in diastolic BP in the DM-group (median increase: 4 mmHg), as compared to the C group. Sub analysis of the DM-group showed a significant increase in diastolic BP (median increase: 5 mmHg) in the GDM-group, but not in the DM1-group.

3.2.4 Serum glucose values

Serum glucose levels were only measured among the women in the CHASE study. Women with a present known diagnose of DM (eg. DM1) were excluded from taking the oral glucose challenge test, and were also excluded from statistical comparisons of fasting serum glucose levels between the groups. As presented in table 2, fasting glucose levels were significantly higher in the GDM-group (median value: 5.5 mmol/l) compared to the PE-group (median value 4.8 mmol/l), but not compared to the C-group (median value: 4.9 mmol/l). No significant differences were found between any of the groups regarding the 2 hour value (table 2).

3.2.5 Cut-off values for overweight, obesity, hypertension and elevated serum glucose

At inclusion in the CHASE clinical study, significantly more women in the DM-group (50 %) had a BMI ≥30 as compared to the C-group (0 %) and the PE-group (11 %) (table 4). Also, significantly more women in the DM-group had a waist to hip ratio ≥ 0.85 as compared to the C-group (92.9 % vs. 33.3 %). The proportions of women in each group having a waist circumference ≥ 88 cm and a WHR ≥ 0.85 are depicted in figure 2.

In the GDM-group two women had a fasting serum glucose value ≥ 6.1 mmol/l (25 %), and one of these women also had a 2-hour value ≥ 7.8 mmol/l. None of the women in the PE- or C-group had abnormal serum glucose values (table 4).

In the PE-group 5 women (27.8 %) had a systolic BP ≥ 130 mmHg, while 2 women (11.1 %) had a diastolic BP ≥ 85 mmHg. In the DM-group, 3 women (21.4 %) had a systolic BP ≥ 130 mmHg and a diastolic BP ≥ 85 mmHg. One of these women with previous GDM and an elevated BP in the CHASE study had a diagnosis of essential hypertension already at time of inclusion in the biobank study in 2001-2004. None of the women in the C-group were classified as presently having hypertension (hypertension was an exclusion criteria in the index pregnancy for the control group). However, these differences in BP between the study groups at the time of CHASE inclusion, were non-significant (table 4).

Table 1: Clinical characteristics of the women before/during early pregnancy and at delivery

	C			PE			DM			C /PE	C /DM	PE/DM
Variable	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**P-value#**	**P-value#**	**P-value#**
Age (years) at delivery	9	33.0	28.5, 36.0	18	30.5	27.0, 33.0	16	32	29.0, 35.7	0.347	0.347	0.760
Pre-pregnancy or early pregnancy weight (kg)	9	69.0	63.5, 77.5	18	65.9	56.5, 72.2	16	72.5	59.0, 94.2	0.433	0.559	0.126
Pre-pregnancy or early pregnancy BMI (kg/m2)	9	22.5	22.0, 25.7	17	21.9	20.5, 25.1	16	25.7	21.7, 30.6	0.367	0.229	**0.049***
Systolic BP (mmHg) before gestational week 20	9	110.0	110.0, 117.5	18	112.5	110.0, 120.0	16	117.5	110.0, 129.5	0.750	0.185	0.215
Diastolic BP (mmHg) before gestational week 20	9	70.0	62.5, 71.5	18	67.5	62.5, 70.0	16	70.0	62.7, 81.0	0.542	0.467	0.175

Mann-Whitney test, * P= significant < 0.05

Table 2: Clinical characteristics of the women at inclusion in CHASE clinical study

	C			PE			DM			C/PE	C/DM	PE/DM
Variable	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**P-value#**	**P-value#**	**P-value#**
Age (years)	9	39.0	36.0, 43.0	18	36.0	34.50, 40.0	14	39.0	36.0, 41.2	0.206	0.704	0.207
Weight (kg)	9	72.0	62.8, 79.8	18	68.8	60.5, 79.5	14	77.7	60.1, 95.9	0.561	0.439	0.220
BMI (kg/m2)	9	24.6	21.5, 26.7	18	24.0	20.7, 27.5	14	27.9	23.6, 34.8	0.860	0.124	**0.025***
Waist (cm)	9	86.0	78.5, 94.2	18	84.5	79.0, 96.3	14	89.2	78.5, 107.7	0.820	0.477	0.470
WHR	9	0.84	0.82, 0.86	18	0.86	0.83, 0.91	14	0,89	0.86, 0.92	0.194	**0.000***	0.149
Systolic BP (mmHg)	9	115.0	109.0, 118.0	18	119.5	115.0, 130.0	14	121.5	117.7, 131.5	**0.047***	**0.010***	0.435
Diastolic BP (mmHg)	9	71.0	70.0, 74.0	18	70.0	68.7, 80.0	14	72.0	69.5, 81.2	0.916	0.524	0.564
Glucose, fasting value (mmol/L) **	8	4.90	4.60, 5.20	18	4.80	4.57, 5.00	8	5.5	4.7, 6.17	0.655	0.138	**0.039***
Glucose, 2 hour value (mmol/L) **	8	4.35	3.80, 4.82	18	4.00	3.47, 4.60	8	4.55	3.60, 6.8	0.419	0.599	0.278

Mann-Whitney test, * P= significant < 0.05, ** women with established DM1 are excluded from the glucose analysis.

Figure 1: Correlation between BMI and waist circumference in women (all study-groups combined) at CHASE inclusion

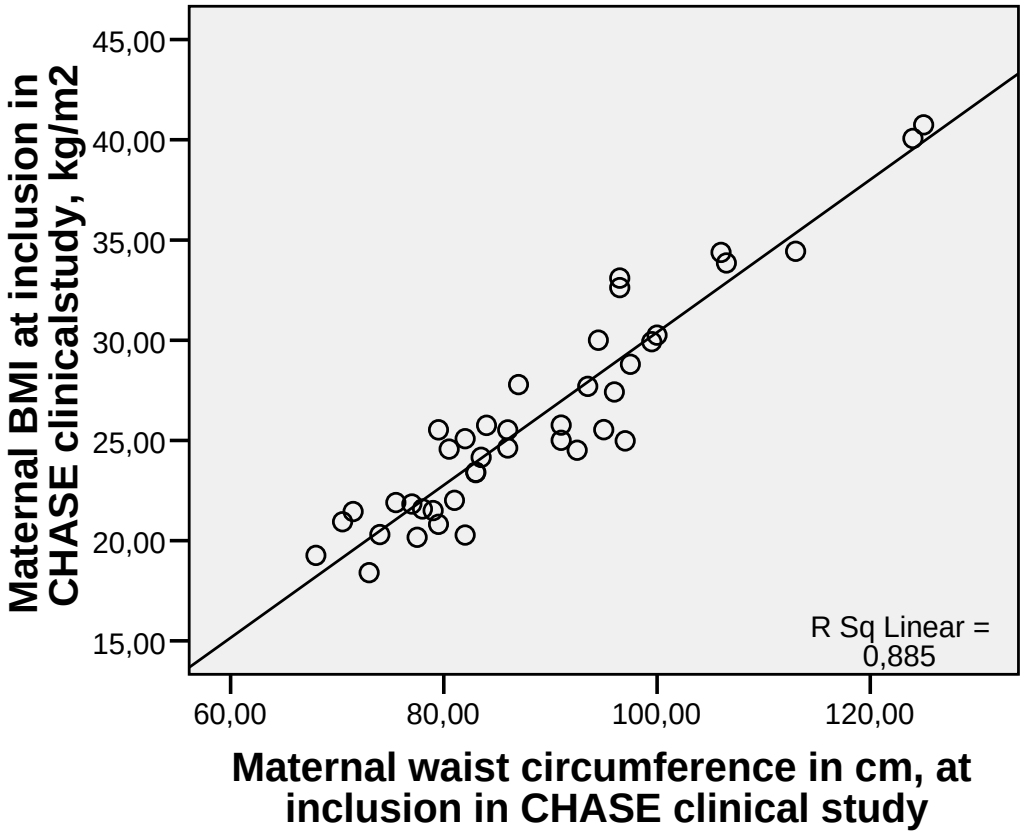


Table 3: Changes in maternal weight, BMI and blood pressure from pre-pregnancy/early pregnancy to inclusion in CHASE clinical study

	C			PE			DM			C	PE	DM
Variable	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**P-value#**	**P-value#**	**P-value#**
Weight (kg)	9	0.0	-2.0, 7.3	18	4.7	-1.4, 9.10	14	3.7	-0.55, 11.1	0.499	**0.022***	**0.020***
BMI (kg/m2)	9	0.0	-0.6, 2.7	17	1.2	-0.6, 3.4	14	1.9	0.6, 3.9	0.594	**0.031***	**0.004***
Systolic BP (mmHg)	9	-2.0	-7.5, 8.5	18	7.0	0.0, 16.2	14	4.0	-0.7, 8.2	0.722	**0.003***	0.074
Diastolic BP (mmHg)	9	1.0	-4.0, 8.5	18	5.0	0.0, 11.2	14	4.0	0.0, 8.0	0.483	**0.004***	**0.050***

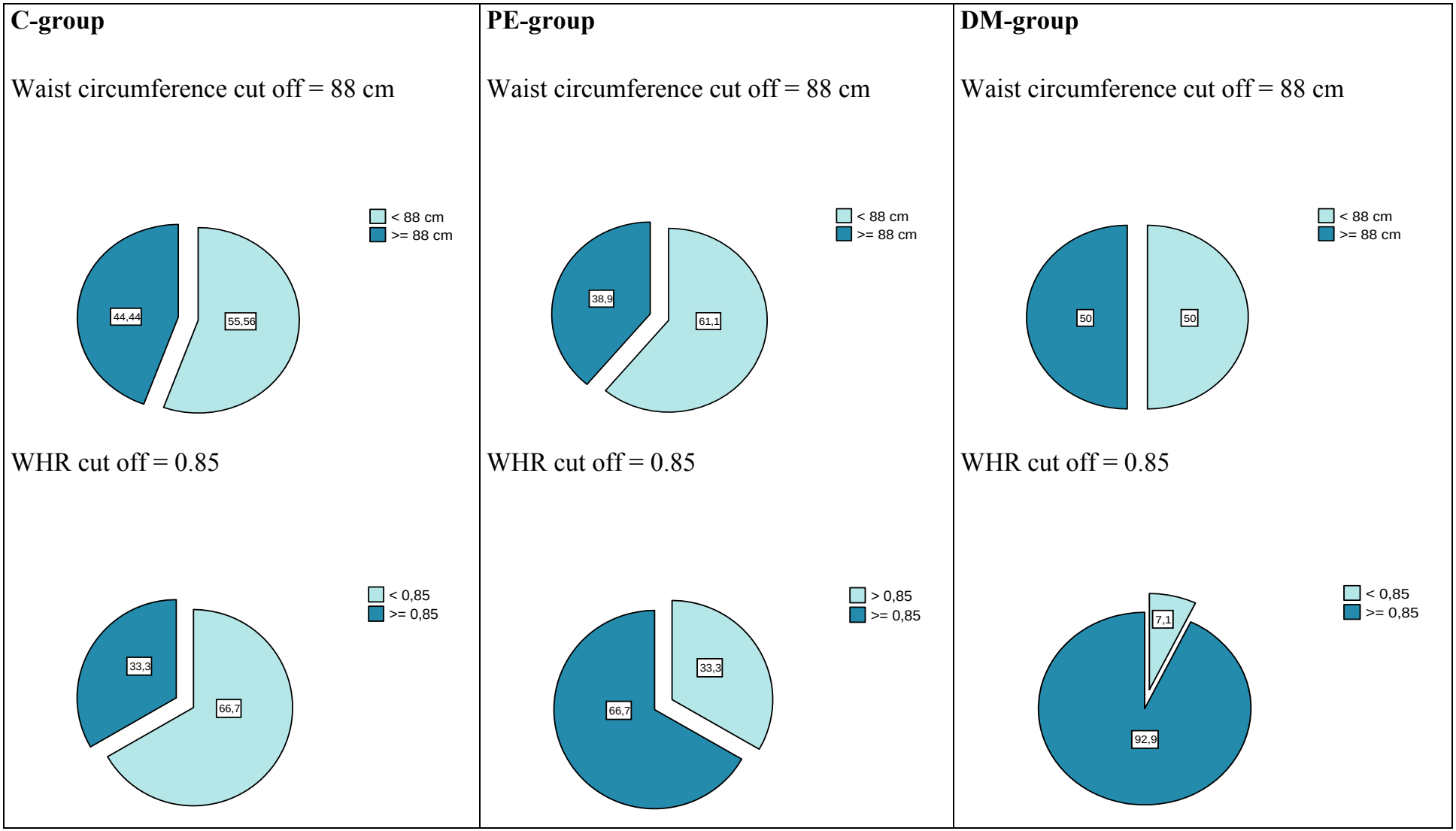
Wilcoxon signed rank test. *P= significant < 0.05.

Table 4 : Proportion of women with values higher than cut-off values for overweight, obesity, hypertension and elevated serum glucose at inclusion in CHASE clinical study

	C			PE			DM			C/PE	C/DM	PE/DM
Variable	**n**	**Frequency**	**%**	**n**	**Frequency**	**%**	**n**	**Frequency**	**%**	**P-value#**	**P-value#**	**P-value#**
BMI (kg/m2) 25.0-29.9 (overweight)	9	4	44.4	18	5	27.8	14	3	21.4	0.395	0.252	0.685
BMI (kg/m2) ≥ 30 (obese)	9	0	0.0	18	2	11.1	14	7	50.0	0.308	**0.013***	**0.017***
Waist circumference ≥ 88 cm (obese)	9	4	44.4	18	7	38.9	14	7	50.0	0.786	0.799	0.536
WHR ≥ 0.85 (obese)	9	3	33.3	18	12	66.7	14	13	92.9	0.107	**0.003***	0.080
Systolic blood pressure ≥ 130 mmHg	9	0	0.0	18	5	27.8	14	3	21.4	0.086	0.145	0.685
Diastolic blood pressure ≥ 85	9	0	0.0	18	2	11.1	14	3	21.4	0.308	0.145	0.433
Glucose, fasting value >6.1**	8	0	0.0	18	0	0.0	8	2	25.0	1.00	0.143	**0.030***
Glucose, 2 hour value > 7.8**	8	0	0.0	18	0	0.0	8	1	12.5	1.00	0.317	0.278

Mann-Whitney test, * P= significant < 0.05, ** women with established DM1 are excluded from the glucose analysis.

Figure 2. Pie charts of the proportions of women in each group having a waist circumference ≥ 88 cm and a WHR ≥ 0.85



3.3 Clinical characteristics of the Children

3.3.1 Age

Median age in years was 6 for all three groups of children, while median age in months was 84 in the C-group, 74.5 in the PE-group and 76.6 in the DM-group of children (table 5). Further sub division of the DM-group into DM1 and GDM showed a statistically significant difference between the children in the DM1-group and the children in the C-group regarding age in months (median values 74 and 84 months respectively).

3.3.2 Anthropometrics

All comparisons of anthropometrical measurements between the main groups of children gave non-significant differences (table 5), but a significantly higher waist to hip ratio was found in the DM1-group compared to the GDM-group (median values: 0.96 vs. 0.92).

3.3.3 Blood pressure

No differences were found in either systolic or diastolic BP between the groups of children (table 5) and all BP were below hypertension thresholds.

3.3.4 Cut-off values for overweight and obesity

A non-significant larger proportion of children in the DM-group was overweight according to overall (BMI) and central (waist circumference) adiposity cut-off values. Only children in the PE- and DM-group were classified as obese according to age- and sex specific BMI (1 and 2 children, respectively) and waist circumference (1 and 1 child, respectively) cut-off values (table 6).

Table 5 : Clinical characteristics of the children at inclusion in CHASE clinical study

	C			PE			DM			C/PE	C/DM	PE/DM
Variable	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**P-value#**	**P-value#**	**P-value#**
Age in years	9	6.0	6.0, 7.0	18	6.0	5.7, 6.2	16	6.0	6.0, 6.7	0.225	0.334	0.744
Age in months	9	84.0	77.5, 89.5	18	74.5	71.0, 83.7	16	76.6	72.2, 83.5	0.075	0.156	0.666
BMI (kg/m2)	9	16.6	14.9, 17.8	18	15.9	14.8, 16.9	16	16.9	14.9, 18.5	0.561	0.677	0.408
Waist in cm	9	59	55.7, 59.7	18	55.7	53.9, 57.6	16	55.2	51.6, 60.5	0.194	0.495	0.849
WHR	9	0.93	0.89, 0.95	18	0.92	0.90, 0.97	16	0.93	0.92, 0.97	0.940	0.610	0.617
WHtR	9	0.47	0.44, 0.48	18	0.46	0.44, 0.50	16	0.46	0.44, 0.50	0.705	0.955	0.629
Systolic blood pressure (mmHg)	9	100.0	90.0, 103.0	17	100.0	95.0, 105.0	16	97.5	90.0, 100.0	0.209	0.600	0.082
Diastolic blood pressure (mmHg)	9	56.0	52.5, 60.0	17	58.0	55.0, 64.0	16	55.0	55.0, 60.0	0.428	0.907	0.404

Mann-Whitney test.

Table 6 : Proportion of children with values higher than cut-off values for overweight and obesity

	C			PE			DM			C/PE	C/DM	PE/DM
Variable	**n**	**Frequency**	**%**	**n**	**Frequency**	**%**	**n**	**Frequency**	**%**	**P-value#**	**P-value#**	**P-value#**
Iso BMI (kg/m2) = 25 (overweight)	9	2	22.2	18	2	11.1	16	6	37.5	0.452	0.441	0.074
Iso BMI (kg/m2) = 30 (obese)	9	0	0.0	18	1	5.6	16	2	12.5	0.820	1.00	0.746
Waist circumference (overweight)	9	1	11.1	18	3	16.7	16	4	25	0.707	0.414	0.555
Waist circumference (obese)	9	0	0	18	1	5.6	16	1	6.3	0.480	0.453	0.933

Mann-Whitney test

3.4 Dietary intake among the women

3.4.1 Consumption of different beverages

There were significant differences regarding consumption of extra low fat milk and skimmed milk between the C-group and the PE-group and between the C-group and the DM-group, with a higher consumption in the C-group (table 7). There were however no significant differences between the C-group and the GDM-group when excluding DM1 cases (data not shown). Regarding alcoholic beverages, women in the PE-group had a slightly higher consumption of beer (median value: 0.1 glass/day) than the C-group (median value: 0 glass/day) and the DM-group (median value: 0 glass/day), while women in the C-group had a higher intake of wine (median value: 0.3 glass/day) compared to the PE- (median value: 0.1 glass/day) and DM-group (median value: 0 glass/day) (table 7). The total intake of alcoholic beverages (beer + wine) did not differ significantly between the groups (data not shown).

There was a non-significant difference (P= 0.052) between the PE-group and the C-group concerning reported intake of sugar containing beverages (median values of 0.1 glass/day and 0.0 glass/day for PE and C respectively) (table 7). The PE-group had a significantly higher consumption of sugar containing beverages than the DM1-group (median values: 0.1 glass/day vs. 0.0 glass/day) and a significantly lower consumption of artificial sweetened beverages compared to the DM1-group (median values: 0.1 glass/day vs. 0.7 glass/day respectively).

Table 7: Consumption of different beverages (glass/day) among the women

	C			PE			DM			C/PE	C/DM	PE/DM
Variable	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**P-value#**	**P-value#**	**P-value#**
Whole milk	8	0.0	0.0, 0.0	17	0.0	0.0, 0.0	13	0.00	0.0, 0.0	0.215	0.433	0.415
Low fat milk	9	0.0	0.0, 1.1	17	0.0	0.0, 1.3	13	0.00	0. 0, 0.1	0.608	0.970	0.492
Extra low fat milk and skimmed milk	9	0.7	0.1, 2.0	17	0.0	0.0, 0.7	14	0.00	0.0, 0.3	**0.035***	**0.034***	0.928
Fruit juice	9	0.3	0.0, 1.3	18	0.3	0.2, 0.7	14	0.1	0.0, 0.1	0.428	0.821	0.139
Sugar sweetened beverages	9	0.0	0.0, 0.1	18	0.1	0.0, 0.3	13	0.0	0.0, 0.7	***0.052***	0.598	0.055
Beverages, artificial sweetened	9	0.3	0.0, 1.3	17	0.1	0.0, 0.5	14	0.7	0.0, 2.0	0.638	0.584	0.200
Beer	9	0.0	0.0, 0.0	18	0.1	0.0, 0.1	14	0.0	0.0, 0.1	**0.015***	0.681	**0.031***
Wine	9	0.3	0.1, 0.5	18	0.1	0.0, 0.1	14	0.0	0.0, 0.3	**0.028***	**0.027***	0.561

Mann-Whitney test, * P= significant < 0.05. N varies due to non-answered questions.

3.4.2 Consumption of different food items

Significant differences in food items between the main groups of women only existed for cod liver oil supplements, with women in the C-group having a higher consumption than women in the DM-group (median values: 0.7 times/day vs. 0 times/day) (table 8). Data on type and amount of bread spreads are not shown .There were however no differences between the study groups. Further subdivision of the DM-group showed that women in the DM1-group had a significantly lower intake of cod-liver oil compared to women in the PE- and C-group (median values: 0.7 times/day, 0.3 times/day and 0 times/day for the C-, PE- and DM1-group respectively), while women in the GDM-group had a significantly lower consumption of cheese (median value:0.3 times/day) compared to the C-group (median value: 1.0 times/day), the PE-group (median value: 1.0 times/day), and the DM1 group (median value: 1.5 times/day) and a significantly lower consumption of meat (median value: 0.3 times/day) than the PE-group (median value: 0.7 times/day).

Table 8: Consumption of different foods (times/day) among the women

	C			PE			DM			C/PE	C/DM	PE/DM
Variable	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**P-value#**	**P-value#**	**P-value#**
Cod liver oil	9	0.7	0.0, 1.0	17	0.3	0.0, 0.7	14	0.0	0.0, 0.2	0.160	**0.016***	0.102
Vitamin supplement	8	0.3	0.0, 1.0	16	0.1	0.0, 0.6	14	0.0	0.0, 0.5	0.596	0.416	0.502
White bread	9	0.0	0.0, 0.1	18	0.0	0.0, 0.1	14	0.1	0.0, 0.1	0.394	0.333	0.790
medium whole grain- and whole grain bread	8	1.1	0.7, 2.0	17	2.1	1.4, 3.0	12	2.0	0.6, 3.0	0.085	0.333	0.625
Cheese	9	1.0	0.7, 1.0	18	1.0	0.7, 1.0	14	0.7	0.3, 1.2	0.823	0.329	0.279
Potatoes	9	0.3	0.3, 0.7	18	0.3	0.3, 0.3	14	0.3	0.1, 0.7	0.518	0.893	0.738
Vegetables	9	1.0	0.7, 1.0	17	1.0	0.7, 1.5	14	0.8	0.7, 1.0	0.643	0.711	0.363
Fruit/berries	9	1.0	0.7, 2.0	18	0.8	0.7, 2.0	14	0.5	0.3, 2.0	0.559	0.346	0.460
Fish	9	0.3	0.2, 0.3	18	0.3	0.2, 0.3	14	0.3	0.1, 0.3	1.000	0.502	0.407
Meat	9	0.3	0.3, 0.5	18	0.7	0.3, 0.7	14	0.3	0.3, 0.7	0.157	0.969	0.145

Mann-Whitney test, * P= significant < 0.05. N varies due to non-answered questions.

3.5 Dietary intake among the children

3.5.1 Consumption of different beverages

The children in the C-group had a significantly higher consumption of extra low fat milk compared to the PE-group (table 9) and the DM1-subgroup (data not shown). In contrast, the children in the PE-group had a higher consumption of low fat milk compared to the C-group. No other differences were found in beverage consumption between the children, although we found a non-significant lower intake of orange juice among the children in the DM-group compared to the C-group (P= 0.073) (table 9).

Table 9: Consumption of different beverages (glass/day) among the children

	C			PE			DM			C/PE	C/DM	PE/DM
Variable	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**P-value#**	**P-value#**	**P-value#**
Whole milk	8	0.0	0.0, 0.5	18	0.0	0.0, 0.7	16	0.0	0.0, 0.2	0.373	0.819	0.463
Low fat milk	9	0.0	0.0, 0.7	17	2.0	0.2, 2.0	15	0.7	0.0, 0.7	**0.018***	0.317	0.090
Extra low fat milk	8	0.7	0.0, 2.0	15	0.0	0.0, 0.1	16	0.0	0.0, 1.6	**0.034***	0.165	0.459
Skimmed milk	7	0.0	0.0, 2.0	15	0.0	0.0, 0.0	16	0.0	0.0, 0.0	0.076	0.153	0.643
Orange juice	9	0.3	0.1, 1.3	18	0.3	0.0, 0.7	15	0.1	0.0, 0.3	0.752	0.073	0.114
Sugar sweetened beverages	7	0.4	0.0, 0.7	17	0.4	0.1, 0.7	16	0.3	0.1, 0.5	0.501	0.866	0.491
Artificial sweetened beverages	8	0.1	0.0, 0.3	15	0.1	0.1, 0.4	16	0.1	0.1, 0.3	0.469	0.553	0.920

Mann-Whitney test, * P= significant < 0.05. N varies due to non-answered questions.

3.5.2 Consumption of different food items

Children in the C-group had a higher consumption of cod liver oil compared to children in the PE-group (median values: 0.7 times/day vs. 0 times/day) (table 10).

Sub-group analyses of the DM-group showed that children in the DM1-group had a significantly lower consumption of fruit and berries (median value: 1.0 times/day) compared to the children in the C-group (median value: 2.0 times/day). No other significant differences between the study-groups were detected.

Table 10: Consumption of different foods (times/day) among the children

	C			PE			DM			C/PE	C/DM	PE/DM
Variable	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**P-value#**	**P-value#**	**P-value#**
Boiled potatoes	9	0.3	0.2, 0.3	18	0.3	0.2, 0.3	16	0.3	0.3, 0.6	0.798	0.320	0.134
French fries	9	0.0	0.0, 0.1	17	0.1	0.0, 0.1	16	0.1	0.0, 0.1	0.109	0.134	0.923
Vegetables	9	0.7	0.7, 1.0	18	0.7	0.2, 1.0	16	0.8	0.4, 1.0	0.391	0.859	0.321
Fruit/berries	9	2.0	1.0, 2.0	17	1.0	0.7, 2.0	16	1.0	0.4, 1.7	0.188	0.070	0.398
Whole grain bread	9	2.0	1.0, 2.5	18	2.0	1.0, 2.2	16	2.0	0.8, 3.0	0.655	0.907	0.762
Fish	9	0.3	0.2, 0.3	18	0.3	0.1, 0.3	16	0.3	0.1, 0.3	0.761	0.636	0.827
Pizza	9	0.1	0.1, 0.3	18	0.1	0.1, 0.1	16	0.1	0.1, 0.1	0.266	0.329	0.926
Hamburger/hot dogs/kebab	9	0.1	0. 0, 0.2	18	0.1	0.1, 0.1	16	0.1	0.0, 0.1	0.622	0.852	0.738
Sweets	9	0.3	0.2, 0.3	18	0.3	0.1, 0.3	16	0.3	0.1, 0.3	0.559	0.819	0.696
Chocolate	9	0.3	0.1, 0.3	18	0.3	0.1, 0.3	16	0.3	0.1, 0.3	0.631	0.899	0.498
Potato chips etc.	9	0.1	0.0, 0.3	18	0.1	0.1, 0.1	16	0.1	0.1, 0.2	0.819	1.000	0.750
Peanuts	9	0.0	0.0, 0.1	17	0.0	0.0, 0.1	16	0.0	0.0, 0.1	1.000	0.916	0.911
Cod liver oil/cod liver oil capsules	9	0.7	0.0, 1. 0	17	0.0	0.0, 0.7	16	0.0	0.0, 0.6	**0.040***	0.082	0.952
vitamins	9	0.0	0.0, 1.0	17	0.3	0.0, 0.7	16	0.1	0.0, 1.0	1.000	0.846	0.955

Mann-Whitney test, * P= significant < 0.05.N varies due to non-answered questions.

3.6 Fruit and vegetable consumption among the women and children according to the Norwegian recommendations

In addition to separate analysis of the responses regarding fruit and vegetable intake, these variables were merged according to the Norwegian dietary recommendations of 2 portions of fruits and berries and 3 portions of vegetables. The proportions of women and children following the fruit and vegetable recommendations are shown in table 11 and 12 respectively.

More women and children in the C-group followed the fruit dietary recommendations (two portions of fruits and berries) than in the other study groups; 44.4 % of the women and 77.8 % of the children in the C-group, 33.3 % of the women and 41.2 % of the children in the PE-group and 35.7 % of the women and 26.1 % of the children in the DM-group. These differences were only statistically significant between the children in the C-group and DM-group (P=0.017). Except for one woman in the DM-group, none of the women and children in any of the groups reported following the recommended intake of 3 portions of vegetables a day.

Table 11: Fruit and vegetable intake among the women in the CHASE study compared to the Norwegian recommendations of 3 or more portions of vegetables and 2 or more portions of fruit a day

	C			PE			DM			C/PE	C/DM	PE/DM
Variable	**n**	**Frequency**	**%**	**n**	**Frequency**	**%**	**n**	**Frequency**	**%**	**P-value#**	**P-value#**	**P-value#**
Fruits (included fruit juice/berries) ≥ 2 portions a day	9	4	44.4	18	6	33.3	14	5	35.7	0.580	0.682	0.890
Vegetables (included potatoes) ≥ 3 portions a day	9	0	0.0	17	0	0.0	14	1	7.1	1.00	0.423	0.270

Mann-Whitney test. N varies due to non-answered questions.

Table 12: Fruit and vegetable intake among the children in the CHASE study compared to the Norwegian recommendations of 3 or more portions of vegetables and 2 or more portions of fruit a day

	C			PE			DM			C/PE	C/DM	PE/DM
Variable	**n**	**Frequency**	**%**	**n**	**Frequency**	**%**	**n**	**Frequency**	**%**	**P-value#**	**P-value#**	**P-value#**
Fruits (included fruit juices/berries ≥ 2 portions a day	9	7	77.8	17	7	41.2	15	4	26.7	0.081	**0.017***	0.396
Vegetables (included potatoes) ≥ 3 portions a day	9	0	0.0	18	0	0.0	16	0	0.0	1.00	1.00	1.00

Mann-Whitney test, * P= significant < 0.05. N varies due to non-answered questions.

3.7 Physical activity in the women

We found significant differences between the women in the C-group and the PE-group, and between the C-group and the DM-group in how often they performed high intensity physical activity for a minimum of 20 minutes continuously during the last seven days. The women in the C-group reported to do it more frequently than women in the other groups (median values: 2 times, 0.5 times and 0 times last seven days for the C-group, PE-group and DM-group respectively) (table 13). The difference between the C-group and the DM-group also remained statistically significant when comparing C-group and the two sub groups of the DM-group separately (GDM and DM1) (data not shown).

The C-group also reported more often performing moderate physical activity in their spare time for a total of at least 30 minutes than the DM-group (table 13). When compared to each of the DM-groups separately, the difference to the C-group was only significant for the GDM-group (data not shown).

When all the questions about maternal physical activity were summarized, median values of total hours spent in physical activity were 3.5, 2.7 and 1.8 in the C-, PE- and DM-group respectively. The difference between the C- and DM-group was statistically significant (table 13).

The women were also asked if the last seven days were representative for their usual physical activity level. 77.8 % in both the C-group and the PE-group, and 50 % in the DM-group reported a representative week, while 22.2 %, 16.7 % and 50 % of the women in the C-, PE- and DM-group respectively reported to be less physical active than usual. These differences were not statistically significant.

Table 13: Physical activity among the women in the CHASE study

Variable	C			PE			DM			C/PE	C/DM	PE/DM
	n	Median	Percentile 25, 75	n	Median	Percentile 25, 75	n	Median	Percentile 25, 75	P-value#	P-value#	P-value#
Walking/bicycling (moderate pace) to or from job/store etc for min. 30 minutes total. How many days of the last seven days?	8	3.0	2.0, 6.0	18	3.0	0.7, 5.2	13	2.0	0.0, 5.0	0.866	0.210	0.370
Spare time physical activity, moderate pace, for min. 30 minutes total. How many days of the last seven days?	8	2.0	2.0, 3. 0	18	2.0	1.0, 3.0	13	1.0	0.0, 1.5	0.238	**0.021***	0.075
High intensity physical activity for min. 20 minutes continuous. How many days of the last seven days?	9	2.0	2.0, 3.0	18	0.5	0.0, 2.0	13	0.0	0.0, 1.0	**0.011***	**0.001***	0.226
Sum of physical activity during the last 7 days (hours).	7	3.5	3.2, 5.5	18	2.7	1.4, 3.9	13	1.8	0.0, 3.0	0.203	**0.035***	0.081

Mann-Whitney test, * P= significant < 0.05. N varies due to non-answered questions.

3.8 Physical activity in the children

No differences in reported physical activity were found between the main children study groups (table 14). There was however a statistically significant more frequent physical activity in the C-group as compared to the DM1-subgroup (median values: 2.5 times/week and 1.7 times/week respectively).

Hours spent on watching television, playing computer or video games etc. did not differ between any of the groups of children (median 0.75 h per day in all study groups) (table 14).

Table 14: Spare time physical activity and inactivity/screentime among the children in the CHASE study

	C			PE			DM			C/PE	C/DM	PE/DM
Variable	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**n**	**Median**	**Percentile 25, 75**	**P-value#**	**P-value#**	**P-value#**
High intensity activity, times/ week	9	2.5	2.5, 5.0	18	2.5	1.0, 5.0	15	2.5	1.0, 2.5	0.490	1.000	0.314
High intensity activity, hours/week	9	2.5	1.7, 2.0	18	2.5	1.0, 3.1	15	1.0	1.0, 2.5	0.802	0.107	0.169
Screen time, hours/day	9	0.75	0.75, 1.62	18	0.75	0.75, 2.50	15	0.75	0.75, 2.50	0.642	0.366	0.583

Mann-hitney test.

4. Discussion

4.1 Design and study power

A scientific weakness of the study is its small sample size, as a small-sized study can give low statistical power in some of the study variables. Because we are not aware of studies with a similar design, it was difficult to carry out any power calculations prior to the CHASE follow-up study, which itself must be regarded as a pilot study.

The major advantage of the CHASE study is its longitudinal design, making it possible to follow the same women and children from delivery to several years postpartum. In this way, each woman, each child and each study group of women and children serves as their own control measure, as most biomarkers can be measured both in biological samples at delivery and 5-8 years postpartum. However, information regarding diet and physical activity has only been obtained during the CHASE follow-up study, making the quality of this part of the study more vulnerable to sample size. This must be taken into consideration when interpreting the results.

4.2 The study population

Of the 156 women invited to participate, 43 women (27.6 %) accepted the invitation. One woman with DM1 and one woman with prior GDM were excluded due to current pregnancy. All of the 43 children were still included, although the total number of women was reduced to 41. This is a comparatively small number of subjects, and it is therefore uncertain if these women and children are representative for the population in question. Especially the number of control subjects is small (n=9 women and 9 children) compared to the DM-group (n=14 women and 16 children) and the PE-group (n=18 women and 18 children), with only half the number of subjects compared to latter group. This is not surprising, as women in the C-group may not have the same personal interest in participating as one might think women

with prior complications in pregnancy may have. I.e. women with pregnancies complicated by PE or DM may be more worried about their own future health and perhaps also future pregnancies, as well as their offspring's future health, compared to women without such complications during pregnancy. However, population characteristics across the groups were quite consistent in terms of age, length of follow up from index pregnancy to CHASE clinical study, ethnicity and maternal educational level. Also, women recruited to the original biobank study in the index pregnancy were not necessarily representative for the whole PE, DM or C group of pregnant women. For women with PE, the necessity of performing a caesarean section, which was decided independent of their study participation, indicate that these women have more severe clinical preeclampsia than the general preeclamptic population. Even if our preeclampsia group is not representative for all women with preeclampsia, we have recruited the group with potentially greatest research interest, as early and severe PE is associated with the largest health consequences for the offspring (139). The advantages of such a biobank outnumber the disadvantages, as no other methodology would ensure similar biobank material from pregnancies not having undergone labour, including maternal and fetal blood and DNA, amniotic fluid, as well as placenta/decidual, fat and muscle tissues.

4.3 Methodological considerations

4.3.1 Blood pressure and anthropometrical measurements

Methods regarding BP readings and anthropometrical measurements were standardized before the start of the clinical study (as described under "methods"). A paediatrician measured the children's BP, while the master student measured the women's BP and performed all the anthropometrical measurements in both women and children. Despite of standardized techniques, it cannot be excluded that measurement errors may have occurred. In the case of any errors or imprecise measurements, it is likely that these would be systematic and thus similar to all groups, as the measurements were performed by the same persons.

4.3.2 Diet and physical activity questionnaire

Numerous methods are available to study an individual's habitual dietary intake, such as weighed food records (WFR), diet diaries (DD)/ Food diaries, 24-hour recalls (24HR) and FFQs. WFR is an extensive method where all foods and beverages consumed by an individual during a day are measured on scales. This method seems to have the best correlation to objective biomarkers of dietary intake, and thus considered the most valid of these dietary assessment tools. As with WFR, DD is a prospective and extensive dietary assessment method. In this method however, each day's dietary intake is estimated on the background of unit portions and/or photos or illustrations of portion sizes. The length of these registrations (WFR and DD) varies, but typically covers several days, weekdays and weekends included. In contrast to the prospective methods, 24HR is a recollection of the participant's dietary intake during the last 24 hours and is typically interviewer-administered. Repeated 24HR likely reduces day-to-day variability that is easily lost with a single 24HR, and is better correlated to biomarkers of dietary intake than is a single 24HR. Another retrospective instrument is the FFQ. This method is shown to be weaker associated with biomarkers of dietary intake than the other methods, but is cost-effective and is a commonly used tool to assess dietary intake in large population studies. These questionnaires can be long or short, depending on the purpose of the study. If the questionnaire addresses the amount of each food item consumed, in addition to frequency, the FFQ is defined as semi-quantitative (136). In the CHASE study we have used short FFQs developed and validated at the Department of nutrition, University of Oslo, Norway. These questionnaires only cover some indicator food items and not the whole diet, and the frequency alternatives are limited. In this way, results may be somewhat imprecise, seasonal variation is easily lost and nutrient content and total caloric intake are impossible to estimate. However, validation studies have demonstrated a significant correlation between increasing intake measured with a four-day precoded food diary and the FFQ used among the children in our CHASE study, and a seven day precoded food diary and the FFQ used among the women in our CHASE study respectively (87, 135). In the children's questionnaire, beverages, fruit and vegetables had the highest correlations to the

precoded food diary. Infrequently eaten food items, such as pizza and potato chips, had weaker correlations. In the women's questionnaire, ten out of sixteen food items had a correlation to the precoded food diary of at least r = 0.5. The questions concerning meat for dinner and the consumption of vegetables in the women's questionnaire had the weakest correlation to the precoded food diary. The simple FFQs used in the present CHASE study are also easy to complete and much less time consuming than the more extensive registrations. Because of the comprehensive clinical examination of both mother and child in the CHASE-follow-up study, these limited questionnaires were our preferred method in order to reduce any burden such reporting of diet may cause on the participants, especially the mother, as most of the children in our CHASE study were too young to administer any dietary registrations on their own. In this way we also believed that compliance to the study recruitment and fulfilling the questionnaires would increase. Due to the small sample sized cohort available, this factor was of high importance when deciding which method would be the most appropriate. Concerning physical activity, indicator questionnaires were likewise also chosen instead of more extensive self reporting methods, or direct measurements of physical activity or physical fitness. Physical activity questionnaire is in general an inexpensive and applicable tool to assess physical activity in large samples. However, the most important limitation of this method is its subjective component, and results may be prone to over-or underestimation. Questionnaires are also less suited for children, due to the complexity in answering these forms. Thus, the women in our CHASE study completed both their own and their child's questionnaire about diet and physical activity. Alternative methods for physical activity assessment include activity monitors such as pedometers and accelerometers, and heart rate monitoring. Methods such as indirect calorimetry and the doubly labelled water method are less applicable on a large study cohort. Accelerometer-based monitors are considered valid in estimating overall physical activity, but are inappropriate in monitoring complex movements, upper body movements, cycling or movements on a graded terrain. Heart rate monitoring is an indirect measure of physical activity, but is only valid for moderate- to vigorous activities, as the relationship between heart rate and oxygen consumption is not linear during rest or

low-intensity activities, and is easily confounded by factors such as caffeine, smoking, stress and other factors not related to physical activity (137).

4.3.3 Statistical analyses

All data were analysed using non-parametric tests, such as the Mann-Whitney U test and the Wilcoxon signed rank test. However, a few of the continuous variables showed a normal distribution, and were initially analysed with the student t-test. The results didn't differ significantly between the two methods used, and for simplicity we decided to present all data based on non-parametric tests.

A large number of variables have been tested in the present master thesis. When analysing several variables there is a certain risk that significant differences between the groups may occur in some of the study variables simply by chance. Although a lower level of statistical significance could have reduced this risk, for instance using the Bonferroni correction (140), there is also a risk that actual differences between the groups would not have been detected if a lower level of significance was chosen.

4.4 Main findings

4.4.1 Anthropometrics

A relatively large proportion of the women were classified as overweight in all groups according to BMI (44.4 %, 27.8 % and 21.4 % in the C-, PE- and DM-group respectively). However, significant more women in the DM-group (50 %) were classified as obese (BMI $\geq$ 30), compared to 11.1 % in the PE-group and none in the C-group. Data from a larger health survey conducted in five counties in Norway in 2000-2003, showed that 26.6 % and 13.2 % of women 30 years of age were overweight and obese, respectively. In the same population, women aged 40 and 45, 32.7 % and 25.5 % were overweight and obese, respectively (94). The proportion of overweight women in our study population therefore resembles these larger

population data, while the proportion of obese in our study differs with a much larger percentage obese women in the DM-group.

In the CHASE clinical study there was no difference in the proportion of women having abdominal obesity when waist circumference was used as an indicator, and waist circumference had a high correlation to BMI. However, when the WHR was compared between the groups, significant more women in the DM-group were identified with abdominal obesity. Also the percentage of women in the PE-group classified as having central obesity increased when the WHR was used as an indicator, while this was the other way around in the C-group of women. This finding is striking, suggesting that fat deposition may be different between these groups, with a more even fat distribution in the C-group of women.

Several studies have elucidated the correlation between overweight and obesity and risk of CVAD. Results have varied regarding which anthropometrical measurement is best at identifying subjects at increased risk. In a cross-sectional survey in 2000, Dalton et al. showed that WHR had the strongest correlations with CVD risk factors before age adjustment in a national sample of 11 247 Australian adults, but after adjustment for age, waist circumference, WHR and BMI had similar correlations with CVD risk factors (95). In 2005, Yusuf et al. conducted a case-control study of approximately 15 000 cases of first myocardial infarction, and nearly 15 000 age- and sex-matched controls. Study participants were enrolled from 52 countries, representing several ethnic groups. They found that BMI and WHR were directly related to risk of myocardial infarction. However, in contrast to BMI, WHR remained a statistically significant risk factor after adjustment for BMI and other risk factors (96). Similar results were obtained by Canoy et al. who prospectively examined the association between body fat distribution and coronary heart disease in a cohort of about 24 500 men and women aged 45-79 years. They found that the abdominal obesity indices; WHR and waist circumference, were more predictive of CHD than BMI. Hip circumference was shown to be inversely related to risk of CHD, after adjustment for waist circumference, BMI, and CHD risk factors (97).

Overweight and obesity are also related to risk of DM2. Both overall obesity as assessed by BMI, and central obesity as assessed by waist circumference and WHR, are associated with increased risk of DM2. Results have also indicated that there may be an additive effect of the combination of overall and central obesity (98-99).

Also, a relationship between adverse pregnancy outcomes and anthropometrical measures has been explored. As reviewed by Siega-Riz et al., there is a clear association between BMI above the normal range and pregnancy complications including pregnancy induced hypertension, PE and GDM (100). However, the distribution of body fat may also play a role. In a cohort of 198 control women and 26 women with PE, Yamamoto et al. showed that WHR, irrespective of overall adiposity, was a significant predictor of the development of PE on stepwise multiple regression analysis (101). Also Soonthornpun et al. found that women with previous PE had higher waist circumferences and WHR than age- and BMI-matched C-subjects, although BMI were not significantly different between the two groups (102).

We also found a statistically significant difference in maternal pre-pregnancy BMI of the index pregnancy between the DM-group and the PE-group, with a higher median BMI in the DM-group (25.7 kg/m2 compared to 21.9 kg/m2), but there was no significant difference between the C-group and the two other groups of women. No significant difference was found between the three groups of women regarding weight gain during pregnancy (data not shown), but during the in-between study period from to the index pregnancy and the inclusion in CHASE clinical study, there was a significant median change in maternal weight (+ 4.7 kg and + 3.7 kg in the PE- and DM-group respectively) and BMI (+ 1.2 and + 1.9 in the PE and DM-group respectively) in both the PE-group and the DM-group, and the difference in BMI between the two groups remained statistically significant. We do not know whether these changes were due to weight gain after the index pregnancy, an excessive retention of gestational weight or both. In a prospective cohort study of 540 healthy adult women with singleton pregnancies, Olson et al. showed that gestational weight gain, postpartum exercise frequency and change in the amount of foods consumed,

were significantly associated with weight change from early pregnancy to 1 year postpartum. These factors were associated with postpartum weight retention as well as major weight gain (103).

In the CHASE clinical study we found that both women with PE or DM during the index pregnancy were less physical active than the C-group of women. No data exists on dietary- and exercise habits before the index pregnancy, and whether there have been any major changes in one or both of these lifestyle factors is unknown. During the postpartum period of 5-8 years, median changes in weight and BMI in our study were small, and the clinical relevance is therefore questionable. If however, such differences attenuates further in the years to come, the differences in risk for CVD between the PE-/DM- and the C-group will increase similarly.

Also a comparatively large proportion of the children examined in the CHASE clinical study was overweight or obese. Similar to the women, more children in the DM-group had a BMI corresponding to overweight and obesity compared to the PE-group and C-group of children. These differences were however not significant. According to age and sex specific BMI cut-off points, 37.5 %, 11.1 % and 22.2 % of the children were classified as overweight in the DM-group, PE-group and C-group respectively, while the proportions classified as obese was 12.5 %, 5.6 % and 0 % for the DM-, PE- and C-group of children respectively. By contrast, the proportion of overweight 9 year old in a nation-wide survey in Norway was 12.8 % and 14.7 % for boys and girls respectively. Likewise, the proportion of obese was 2.8% and 4.7 % for boys and girls respectively (110). Thus, our findings indicate that the prevalence of overweight among children after diabetic pregnancies are higher than in the general population as well.

A BMI in the upper percentiles in adolescence has been related to increased risk of both cause specific mortality, including circulatory system diseases and ischemic heart disease, and total mortality in adulthood. Whether this is connected to long-term obesity is unknown. However, childhood obesity seems to be a predictive factor for adult obesity (105-106).

Overweight and obesity are also highly important risk factors for the development of DM2 in children and adolescents, probably through the association with insulin resistance. If DM2 is diagnosed already during childhood, typical long term complications of DM, such as neuro-, retino- and nephropathy and atherosclerotic CVD, will also develop at earlier stages in life (104).

Similar to adults (130), evidence indicates that children with central obesity have a higher risk of metabolic disturbances such as an unfavourable lipid profile, elevated BP and insulin resistance (128). In the present CHASE study, a non-significant higher proportion of children in the DM- and PE-group were overweight and obese according to waist circumference measurements, compared to the C-group.

4.4.2 Blood pressure

In the CHASE clinical study we found a significant higher maternal median systolic BP in both the PE-group and the DM-group compared to the C-group. Systolic and diastolic hypertension according to ATP-III criteria was identified among a proportion of women in the PE- and the DM-group, but not in the C-group.

Compared to C-subjects, higher BP, either systolic BP or both systolic and diastolic BP post-delivery in women with PE has been described by others. In three similar clinical follow-up studies of BP in women with a history of PE, the mean or median interval between delivery and follow-up was 1-6 years. Mean systolic- and diastolic BP in women with previous PE ranged from 118 to 122 mmHg, and 76-81.5 mmHg respectively among these studies, while mean systolic- and diastolic BP in C-women ranged from 109- to117 mmHg, and 70-74 mmHg respectively (102, 107-108). Smith et al. (108) reported the prevalence of hypertension one year postpartum in the PE-group as 20.3 % with a systolic BP ≥ 130 mmHg and 38.6 % with a diastolic BP ≥ 85 mmHg, while the corresponding numbers were 1.4 % and 5.7 % in the C-group.

Vhor and Boney also found that from 5 to 11 years post-delivery, women with a history of GDM had higher systolic BP than women without a history of GDM, ranging from 121.8 to 124.9 mmHg in women with previous GDM, and from 117 to

118 mmHg in women without previous GDM. They also reported that by 11 years postpartum, 30 % of the women with previous GDM had systolic hypertension (systolic BP ≥ 130 mmHg), compared to 14 % among the controls (109).

These results are consistent to our findings in the CHASE clinical study. Although Smith et al. reported a much higher percentage of diastolic hypertension among women with previous PE compared to our findings, these results may not be quite comparable taken into consideration the differences in years of follow-up from delivery, as well as possible differences in the study population.

4.4.3 Serum glucose levels

In the CHASE clinical follow-up study we identified elevated fasting values of serum glucose in two women (25 %) with previous GDM, indicating abnormal glucose tolerance. One of these two women also had elevated serum glucose values after the 2-hour glucose challenge test, but the levels were not compatible with diabetes. However, it is estimated that approximately 30 % of subjects with abnormal glucose tolerance after 10 years will develop DM2 (133).

A study by Lauenborg et al., investigating the prevalence of the metabolic syndrome in a large Danish cohort of women with previous diet treated GDM and women with uncomplicated pregnancies at median 9.8 years of follow-up (4-23 years), detected impaired fasting glucose among 11.0 % of the previous GDM-group, and 4.3 % in the comparator group. The total prevalence of glucose intolerance in the GDM group was 67.6 %, of which 39.9 % had established DM, compared to 19.2 % and 3.3 % respectively in the comparator group. Median follow-up in our CHASE study was 6.5 years (5-8 years), and it is possible that a longer follow-up time would be more appropriate in detecting the risk of developing glucose intolerance or diabetes postpartum in women with prior GDM. However, Lauenborg et al. have earlier also demonstrated a 40 % incidence of DM in Danish women with a history of diet treated GDM at a median of 6-7 years post-delivery (134). Again, our small-sample sized study must be taken into consideration when interpreting these results.

Even though we did not find any differences in median values of serum glucose levels between either of the two pregnancy complication groups and the C-group, we cannot exclude that there could be differences in insulin levels between the groups. Normal serum glucose values in women with a history of PE have been reported by others, despite concomitant findings of elevated serum insulin levels (107-108). Serum insulin measurement will be performed later in the CHASE study, following completed patient inclusion and data collection.

4.4.4 Dietary intake

To our knowledge there are few if any publications regarding dietary intake in women and their offspring several years after pregnancies complicated by PE or DM. Based on the epidemiological increased risk of developing CVAD after such complications in pregnancy, we were especially interested to find out whether there are any indications that diet may be a contributing factor.

Our Chase study results showed a little variation in reported dietary intake across the groups of women and children, with a few exceptions. The most striking findings were differences in the consumption of sugar-containing soft drinks, lean milk types, cod liver oil and fruit/berries.

Sugar-sweetened soft drinks

Our results showed that women in the PE-group had a significant higher consumption of sugar-sweetened beverages compared to the DM1-subgroup of women and a borderline significant higher consumption compared to the C-group.

Clausen et al. also found that a higher intake of sucrose during the second trimester of pregnancy was associated with increased risk of PE. Among the women with a high intake of sucrose, sugar-sweetened soft drinks accounted for nearly 70 % of the total sucrose intake, and sugar-sweetened soft drinks was independently related to the risk of PE-development. The authors suggested that the association between a high intake of sucrose and the increased risk of PE could be due to the suppressive effect of

hyperglycemia on endothelium-dependent vasodilation, or by a worsening of the dyslipidemia, which is also associated with endothelial dysfunction (111). Our finding, which indicates that the postpartum intake of sugar-sweetened soft drinks is higher among women with previous PE compared to C-subjects, is therefore interesting, as endothelial dysfunction is also linked to the pathogenesis of CVD and DM.

Sugar-sweetened soft drinks is one of the major sources of added sugar in the Norwegian diet, and a high consumption of these types of beverages may also be a contributing factor in the development of overweight and obesity (112). Some evidence indicate that energy consumed as liquid is less satiating as energy consumed as solid food (47), and this could be a likely explanation. A reduction in the consumption of sugar-sweetened soft drinks is therefore recommended and highly desirable (112).

Despite the differences observed in our study, the amounts consumed by the PE-group are relatively small, corresponding to less than a glass a week. Underreporting is a general problem of self-reported dietary intake such as FFQs (127), and the actual intake could theoretically be somewhat higher. We do not know whether this underreporting, if present, should differ between the study groups. We found no differences in educational level between the study groups, which could also theoretically influence the reporting patterns of dietary intake and physical activity.

In contrast, compared to women in the PE-group, women in the DM1-group reported a higher intake of artificial sweetened soft drinks. A likely explanation for these differences between the PE- and DM1-group, is that DM1-subjects, due to their underlying disease and need for insulin treatment, avoids food items with a high glycemic index, such as sugar containing soft drinks.

Milk consumption

Women in the C-group reported a significant higher intake of lean milk types (extra low fat- and skimmed milk) compared to the two other groups of women. Also the

children in the C-group had a significant higher intake of extra low fat milk compared to the children in the PE-group and the DM1-subgroup of children. Because of the high percentage of saturated fat in cow's milk, it is generally recommended to choose lean milk types. The women and children with a lower intake of extra low fat- or skimmed milk did not replace this lower consumption with whole fat milk type, but children belonging to the PE-group had a significantly higher intake of low fat (1.5 % fat) milk as compared to the C-group.

Several studies have investigated the possible protective effect of milk and dairy products on hypertension and stroke incidence, with some, but not all, showing an inverse relationship. Dietary minerals such as Ca, Mg and K, as well as milk peptides, have been suggested to exert antihypertensive effects, but the knowledge in this area is still limited (113-115). In the DASH-diet (Dietary Approaches to Stop Hypertension) guidelines, advice to increase the consumption of low fat dairy products is included (55).

Also, the potential protective effect of calcium on PE prevention has been investigated in several studies (116-117). Results from these studies are however inconsistent, and the issue seems to be controversial.

Cod liver oil supplement

In the present study we found that women in the C-group had a significantly higher consumption of cod liver oil supplement compared to women in the DM-group, while comparisons between the children showed a statistically significant higher intake of cod liver oil in the C-group compared to the PE-group.

Cod liver oil contains long chain n-3 fatty acids. Long chain n-3 fatty acids have been shown to exert beneficial effects on CVD risk factors, such as reducing plasma levels of TAGs, BP, platelet aggregation and inflammation, improving endothelial function, lipoprotein profile and have also been related to a decreased risk of CVD and cardiovascular mortality in both epidemiological- and intervention studies (118-120).

Therefore, it is of interest that the potentially beneficial cod liver oil is more often consumed by women and children with the lowest risk of CVD (the control group).

Along with fish oil supplements, such as cod liver oil, oily fish is the main dietary source of long chain n-3 fatty acids in the Norwegian diet. We didn't find any differences in fish consumption among the groups in the CHASE study. We do not know however, if there are any differences in the consumption of oily fish among the groups, as the question about fish consumption was not sub-divided into oily- and lean fish in the FFQs. Also, the question about fish consumption only covered fish for dinner, not fish used on sandwiches or in other form. Hence, although the use of cod liver oil supplements was different between the groups, it is difficult to conclude whether there are any significant differences in the total intake of long chain n-3 fatty acid.

Fruit and berries

A non-significant higher proportion of women and children in the C-group followed the Norwegian dietary recommendation of two or more portions of fruits and berries a day compared to the other CHASE study-groups. However, with the exception of the C-group of children, where nearly 78 % consumed the recommended amounts, less than 50 % of the women and children within each group followed these guidelines. Children in the DM-group had the less frequent consumption of fruit and berries, and the difference to the C-group was statistically significant.

Using data from a nation-wide survey in Norway ("UNGKOST 2000"), Frost Andersen et al. showed that children and adolescents (four-year-olds and pupils in the fourth and eighth grades) consumed less than half the recommended intake of fruit and vegetables. Results from this survey also showed that total diet quality increased with increasing intake of fruit and vegetables. A positive correlation was found between fruit and vegetable intake and dietary content of all vitamins and minerals and dietary fiber, as well as a negative correlation between fruit and vegetable intake and energy content from sugar and saturated fat (88). Because of the limitations of the FFQs used in our study, such correlation analyses could not be performed.

However, the children's questionnaire used in the present CHASE study was validated against a pre-coded food diary in the same study ("Ungkost 2000"), and fruit and vegetables were among the food items that showed the best correlations to the pre-coded food diary. It is therefore likely that the quality of the diet is decreased among the children with low intakes and increased among the children with higher intakes in our CHASE study as well. Similar correlations between fruit and vegetable intake and diet quality were also found in a study by Dennison et al., who examined the fruit and vegetable intake among 168 healthy US children 2 and 5 years of age (121).

A recent publication by Holt et al. also showed that fruit and vegetable consumption was inversely related to markers of inflammation and oxidative stress in youth. This association has previously been reported in adults as well (122).

4.4.5 Physical activity

In the present CHASE clinical study, we found that women in the C-group more often performed high-intensity physical activity compared to women in the PE- and DM-group. Women in the DM-group were the less physical active of the three study-groups, also regarding moderate-intensity activity.

Physical activity confers a wide range of health benefits and is central to the prevention and treatment of obesity, hypertension and other CVAD (63, 123-124). Interestingly, in the CHASE study, women in the PE-group and the DM-group also had higher systolic BP compared to women in the C-group, and women belonging to the DM-group were more obese compared to women in the C-group. However, physical activity is also inversely associated with cardiovascular- and all-cause mortality in both men and women independent of other risk factors (125).

Evidence also indicates that physical activity before and during pregnancy may lower the risk of developing pregnancy complications such as GDM and PE. Physical activity, through means of pre-pregnancy weight reduction and the prevention of excessive weight gain during pregnancy, may also reduce the risk of other obesity

related complications of pregnancy, such as delivery of large for gestational age- and macrosomic babies, caesarean section and instrumental delivery (126). Because of the increased recurrence risk in women with previous GDM and PE, increased postpartum exercise could possibly have a beneficial impact on subsequent pregnancies as well.

5. Conclusion

1. Anthropometrics

Preliminary results of the CHASE study have identified a higher obesity rate among women in the DM-group, as assessed by BMI and WHR, compared to the other CHASE study-groups and women in the general population. There were no differences in waist circumference between the women study-groups. Although we did not find any statistical significant differences in any of the anthropometrical measurements among the children, a comparatively high proportion of children of diabetic pregnancies was classified as overweight according to age- and sex-specific BMI and waist circumference calculations.

2. Blood pressure

The CHASE study further demonstrated that women with PE or DM in a pregnancy 5-8 years ago have higher systolic BP, but not diastolic BP, as compared to control subjects of the same study cohort. There were however no differences in BP between the groups of children included.

3. Serum glucose

There were no differences in median values of serum glucose, neither in the fasting state, nor 2 hours after an oral glucose challenge test between the C-group and either of the PE- or GDM-group of women in the CHASE study-cohort. Two women with previous GDM were however identified with impaired glucose tolerance and will be subject to follow up by own general practitioner.

4. Dietary intake

The results of the present CHASE study showed a little variation in reported dietary intake across the main groups of women and children, with a few exceptions; differences in the consumption of fruit, lean milk types and cod liver oil supplement (more women and children in the C-group), and sugar-sweetened soft drinks (more women in the PE-group) were the main findings.

5. Physical activity

The CHASE study also demonstrated that women with pregnancies complicated by PE or DM 5-8 years ago, were less physical active compared to women with uncomplicated pregnancies 5-8 years ago. Differences in physical activity or inactivity (screen-time) were not demonstrated among the children study-groups.

Although we did not find major differences in dietary intake among the groups of women or children, it is still likely that women and children in the PE and DM cohort would benefit from improvements in diet. Since they run an epidemiological augmented risk of CVAD as compared to women and children of uneventful pregnancies, it would probably be beneficial if their food intake and physical activity level were at least as good as in the control group, and that the obesity rate was not higher than in the general population (ideally lower). The CHASE study has identified potential intervention possibilities such as weight reduction in the women and prevention of excessive weight gain in the children, increased consumption of fruit- and vegetables, promoting the use of lean milk types rather than higher fat milk types, decreasing the intake of sugar-sweetened beverages and possibly also advice regarding cod liver oil supplement. Women with previous PE or DM in pregnancy should also receive advice to increase physical activity. Whether information strategies after a pregnancy complicated by PE and/or DM is effective in reducing these risk factors for CVD, as well as really reducing the incidence and severity of CVD in the women and their offspring, remains to be explored.

Further studies on the biological samples and endothelial function studies in the total CHASE cohort will identify whether the differences between the study groups in anthropometrics (such as BMI, WHR etc), BP and environmental factors (food intake and physical activity) are associated with differences in circulating biomarkers of endothelial health and function.

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7. Appendixes

1. Information letter to the women invited to the CHASE study with informed consent scheme.
2. Clinical information form.
3. Diet and physical activity questionnaire for the women in CHASE.
4. Diet and physical activity questionnaire for the children in CHASE.
5. Procedure for the oral glucose challenge test.

CHASE – vs 26.10.08 **SIGNERES OG RETURNERES AV STUDIEDELTAKER**

Forespørsel om deltakelse i forskningsprosjektet

"CHASE: cardiovaskulær helse hos mor og barn etter svangerskapskomplikasjoner"

Bakgrunn og hensikt. Dette er et spørsmål til deg om å delta i en forskningsstudie for å forstå mer om sammenhengene mellom svangerskapsforhold og senere helse for mor og barn. Du som spørres om å delta i denne studien, var deltaker i en forskningsstudie på Fødeavdelingen ved Ullevål universitetssykehus for 5-8 år siden, der det ble samlet prøver fra deg og fra barnets navlestrengsblod (det siste dersom du ble keisersnittsforløst). Både kvinner med normale graviditeter, gravide med diabetes og svangerskapsforgifting ble inkludert i studien, og vi fikk tillatelse til å ta kontakt med deg senere for å spørre deg om å delta i en oppfølgingsstudie. Det er Ullevål universitetssykehus som også er ansvarlig for CHASE-studien**.**

Hva innebærer denne studien? Deltakelse i "CHASE"-studien innebærer en helseundersøkelse av deg og ditt barn (det som ble født da du deltok i studien på Ullevål 5-8 år siden), inkludert en blodprøve av dere (barnet får vanlig bedøvelseskrem før blodprøven fra albuevenen). I tillegg vil vi benytte opplysninger fra den forrige studien du var med i. Før undersøkelse på Ullevål fyller du ut et kort kost- og aktivitetskjema for deg selv og barnet ditt (tar ca 10 minutter). Ved undersøkelsen på Ullevål tas blodprøvene, en smertefri ultralydundersøkelse av barnets hjerte (av barnelege, tar ca 15 minutter), samt at blodstrømmen i fingertuppen til barnet og deg selv måles smertefritt (tar ca 15 minutter). Undersøkelse gjøres en dag som passer best for deg, på morgenen før dere spiser frokost. Dere vil få servert frokost etter blodprøvetakingen. For å ha god tid til pauser og lek, setter vi av ca 2 timer til samtale og undersøkelser totalt. Dersom du ønsker tilbakemelding på kosthold, fysisk aktivitet og vanlige helseparametre (blodtrykk, kroppsmasseindex, blodsukker etc), kan du avtale ny samtale (på Ullevål eller per tlf.) med masterstudenten i ernæring tilknyttet prosjektet. Mer informasjon om hva undersøkelsen innebærer, finner du i kapittel A (side 2).

Mulige fordeler og ulemper. Du og barnet ditt vil ikke ha noen spesielle fordeler av å delta i studien, bortsett fra at du får tilbakemelding om vanlige helseparametre og kostholdsveiledning, dersom du ønsker dette. **Dersom du ønsker å delta i studien, innebærer dette praktisk denne undersøkelsen på Ullevål av deg og barnet ditt.** Vi forskere har håp om å finne faktorer som kan være av betydning for forståelse av mekanismer bak helse- og sykdomsutvikling hos mor og barn etter svangerskapskomplikasjoner. Vi håper at økt kunnskap kan bidra til å styrke positiv helseutvikling samt å forebygge sykdom på lang sikt.

Hva skjer med blodprøvene og informasjonen om deg og barnet ditt? Prøvene tatt av deg og ditt barn og informasjonen som registreres om dere skal kun brukes slik som beskrevet i hensikten med studien. Opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter dere til deres opplysninger og prøver gjennom en navneliste. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til dere. Blodprøvene vil oppbevares med kopling til din og barnets identitet på Ullevål inntil de er oppbrukt. Etter senest 20 år vil kopling til din og barnets identitet slettes, og prøver og informasjon oppbevares etter dette anonymt. Vi ber også om å kunne innhente relevante opplysninger fra journalene deres ved UUS og andre sykehus, samt fra offentlige registre, beskrevet i Kapittel B, side 3). Det vil ikke være mulig å identifisere deg eller ditt barn i resultatene av studien når disse publiseres.

Frivillig deltakelse. Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien, uten at det får konsekvenser for behandling av deg eller ditt barn på sykehuset. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på slutten av informasjonsskrivet (Kapittel C, side 3), og sender den tilbake til sykehuset i vedlagte frankerte konvolutt. Vi vil så ta kontakt med deg per telefon for å avtale tid for undersøkelsen. Vi vil kontakte per tlf kvinner som ikke har svart på denne henvendelsen innen 3 uker, for å sikre oss at denne forespørselen er kommet frem.

Mer viktig informasjon om studien finnes i Kapittel A og B (side 2 og 3).

Vennlig hilsen leger/forskere på Kvinneklinikken og Barneklinikken ved Ullevål universitetssykehus:

Overlege dr. med ***Annetine Staff*** *(prosjektleder, gynekolog, førsteamanuensis),*
prosjektkoordinator ***Marie Skogstad****,*
doktorgradsstipendiat ***Anja Lee*** *(barnelege) og masterstudent i ernæring* ***Anne Stine Kvehaugen****.*

Kapittel A- utdypende forklaring om hva studien innebærer

- **Bakgrunnsinformasjon for studien:** Studien forsøker å finne bakenforliggende faktorer som kan forklare hvorfor noen kvinner etter svangerskap har endret risiko (enten lavere eller høyere enn gjennomsnittet) for å utvikle hjerte- og karsykdom i voksen alder. Forskning tyder på at forhold i svangerskapet kan være viktige både for mor og barns helse senere, samt at ernærings- og aktivitetsforhold spiller en rolle. Vi vet at kvinner som har hatt svangerskapsdiabetes eller svangerskapsforgiftning har en litt høyere risiko enn kvinner som har hatt svangerskap uten disse komplikasjonene til å utvikle hjerte- og karsykdom eller diabetes. De aller fleste kvinnene som har gjennomgått disse svangerskapskomplikasjonene utvikler imidlertid IKKE hjerte- og karsykdom eller diabetes i voksen alder. Vi kjenner dårlig til de biologiske mekanismene som har betydning for sammenhengen mellom svangerskapet og senere helse- og sykdomsutvikling. Denne forskningsstudien forsøker å finne noen av disse sammenhengene.
- **Hvem kontaktes for å delta i CHASE-studien?** Ca 160 kvinner kontaktes i dette forskningsprosjektet. Alle kvinnene deltok ila 2001-2004 i en forskningsstudie ledet av førsteamanuensis, overlege, dr. med. Annetine Staff ved Kvinneklinikken, Ullevål universitetssykehus. I den forrige forskningsstudien ga dere oss forskere tillatelse til å kunne ta kontakt ved en senere anledning for en oppfølgingsstudie. CHASE er en slik oppfølgingsstudie, der vi altså på nytt ønsker å rekruttere dere til forskning for å gi svar på viktige helsespørsmål. Fordelen med denne nye undersøkelsen er at vi kan knytte informasjon fra svangerskapet og fødselen med informasjon fra dere flere år etterpå, dvs vi kan følge samme kvinne og barn over tid.
- **Dekking av eventuelle utgifter:** Parkeringsutgifter på Ullevål samt eventuelle reiseutgifter kan avtales refundert når vi kontakter deg (per telefon) for tid til undersøkelsen av deg og barnet ditt.
- **Videre undersøkelser etter CHASE-studien**: Dersom du eller barnet mot formodning skulle vise tegn på sykdom som bør utredes nærmere, slik som høyt blodsukker eller hjertesykdom, så vil vi sørge for videre oppfølging i helsesystemet.
- **Undersøkelser, blodprøver og annet de inkluderte mødrene og barna gjennomgår:**

Før undersøkelsen på Ullevål:

Mor og barn: Et kort kost- og aktivitetsskjema for deg og barnet ditt fylles ut av deg før undersøkelse på Ullevål (tar ca 5-10 minutter). Disse skjemaene sender vi deg når du har sendt inn det signerte samtykket til å delta (Kapittel C, side 3).

Under undersøkelsen på Ullevål (max 2 timer totalt, inkludert pauser og tid til lek):

- Mor og barn: Vekt og høydemåling, innhenting av vanlig klinisk informasjon (sykdom, medikamenter, vektutvikling etc).
- Mor og barn: Det tas blodprøve av deg og barnet ditt (prøven tas etter hudbedøvelse for barnet). Dere skal møte fastende til undersøkelsen, dvs ikke ha spist eller drukket 6 timer før undersøkelsen. Vi foreslår derfor at dere møter til undersøkelsen kl 8, slik at dere kan spise den frokosten dere ønsker hos oss etter at blodprøvene er tatt.
- Bare mor: For de kvinnene som har hatt svangerskapsdiabetes eller svangerskapsforgiftning (preeklampsi), tilbyr vi en blodsukkerbelastning. Denne "sukkerbelastningen" kan avdekke begynnende tegn til diabetesutvikling og diabetes. Dette er rutinetilbud til alle som har hatt svangerskapsdiabetes. Også kvinner med normale svangerskap kan få utført denne testen, hvis de ønsker det. I praksis betyr det at vi legger inn en kanyle i albuevenen slik at vi bare stikker deg en gang. Fra denne kanylen trekker vi blodprøver 2 ganger: første gang når du kommer om morgenen (fastende), så 2 timer etter at du har drukket et glass med sukkervann. Etter dette fjerner vi kanylen, og du kan få spise frokost hos oss.
- Både mor og barn: Blodstrømmen i fingertuppen måles smertefritt med et apparat (tar ca 15 min).
- Bare barn: En barnelege med lang erfaring vil foreta ultralydundersøkelse av barnets hjerte (tar ca 15 minutter), en helt smertefri undersøkelse.

Før, under eller etter undersøkelsen på Ullevål:

- Mor og barn: Dere får 2 glass til urinprøve (1 til deg og 1 til ditt barn). Disse prøvene kan leveres på undersøkelsesdagen eller senere (vi avtaler hva som passer deg best per tlf).
- Mor: Dersom du ønsker tilbakemelding på kosthold, fysisk aktivitet og vanlige helseparametre (blodtrykk, kroppsmasseindex, blodsukker etc), kan du avtale ny samtale (på Ullevål eller per tlf) med ernæringsfysiologi-studenten i prosjektet.

Kapittel B - Personvern, biobank og dine rettigheter

Personvern: Opplysninger som registreres om deg oppbevares slik beskrevet på side 1. Som i forrige undersøkelse du deltok i på Ullevål (som gravid), ønsker vi mulighet til å følge din og barnets helse over tid, ved å få din tillatelse til å kople studieresultater mot forskriftsregulerte registre (Kreftregisteret, Fødselsregisteret, Dødsårsaksregisteret) og mot journaler i andre sykehus, for opplysninger som er relevante for denne studien. Ullevål universitetssykehus (adm. direktør) er databehandlingsansvarlig for dine personopplysninger.
Informasjon til og samtykke fra barnet ditt til å delta i forskningsprosjektet: Siden barnet ditt (det som ble født da du var med på forskningsstudien, ila 2001-2004) er så ungt, vil ditt samtykke for å delta i studien også gjelde for barnet ditt. Vi har et enkelt informasjonsskriv med tegninger om hva som skal gjøres i undersøkelsen tilpasset de eldste barna i studien, som du kan vurdere om ditt barn skal få. Dersom ikke blodprøver er brukt opp når barnet ditt er myndig (18 år), vil vi ta kontakt med deg for at ditt barn selvstendig skal bestemme om vi kan bruke prøver og informasjon videre.
Biobank: Blodprøvene som blir tatt av deg og ditt barn, og informasjonen utledet av dette materialet, vil bli lagret i en forskningsbiobank ved Ullevål universitetssykehus. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Biobankkoordinator Roger Bjugn ved Ullevål universitetssykehus er ansvarlig for biobanken (ansvarshavende), og prosjektleder Annetine Staff kan kontaktes ved evt. spørsmål. Biobanken planlegges å vare til prøvene er oppbrukt. Etter senest 20 år vil materialet og opplysninger bli anonymisert etter interne retningslinjer.
Behandling av materiale og opplysninger hos andre: Hvis du sier ja til å delta i studien, gir du også ditt samtykke til at avidentifiserte prøver og opplysninger sendes til samarbeidende forskere i Norge og i utlandet, som bidrar til CHASE-studiens undersøkelser. Ullevål er fremdeles ansvarlig for prøvene og opplysningene, dersom de sendes til samarbeidende forskere. Opplysninger som identifiserer deg eller ditt barn vil ikke utleveres. Noen land utenfor EU/EØS har lover som ikke tilfredsstiller europeisk personvernlovgivning, men dersom prøver sendes dit, vil ikke opplysninger om helseforhold følge prøvene, sammenkoplingen mellom helseopplysningene og de biologiske prøvene vil skje i Norge. Ved henvendelse til prosjektleder vil du kunne få en oppdatert liste over hvilke samarbeidspartnere dette omfatter.
Rett til innsyn og sletting av opplysninger om deg og sletting av prøver: Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte prosjektleder Annetine Staff (22119800) på Kvinneklinikken, Ullevål.
Forsikring: Alle som deltar i studien er dekket av norsk pasientskadeerstatningsordningen (www.npe.no).
Informasjon om studieresultater: Dersom du ønsker tilbakemelding om kosthold, fysisk aktivitet og helseparametre som kroppsmasseindeks og blodsukker, kan du avtale samtale (på UUS eller per tlf) med ernæringsfysiologi-studenten knyttet til prosjektet, for en samlet gjennomgang.

Kapittel C: Samtykkeerklæring

Samtykkeerklæring: Jeg er villig til å delta i CHASE-studien ----------	Jeg kan enklest kontaktes for avtale om undersøkelsestid på telefon **(mobil og evt fasttelefon nummer):** ----------
(Signert av prosjektdeltaker, dato)	

Dersom du ønsker å vite mer, er det bare å kontakte oss for ytterligere informasjon,
ved prosjektkoordinator **Marie Skogstad** (tlf 93 48 42 47,mail riog@uus.no), Ullevål universitetssykehus.

Dersom du ønsker å delta i undersøkelsen, **vennligst returner dette informasjonsskrivet i signert form i vedlagte frankerte konvolutt. Du beholder selv en kopi av informasjonsskrivet ("Kopi til studiedeltaker").** For å være sikker på at vi har rett kontaktadresse for deg, vil vi forsøke å kontakte deg igjen en stund etter at vi har sendt deg dette brevet, dersom vi ikke har hørt fra deg.

Appendix 2

CHASE-studien 2008-9	UUS UNDERSØKELSESDATO:				
KLINISKE DATA				**CHASE-nummer (CM1-149), nummerering etter us-dato**	
skjema fylles ut av studieansatte: hvem:					
MORS KLISTREMERKE				**MORS NAVN OG FØDSELSNUMMER**	
Mors diagnose (sett ring) ved inklusjon i sectiobiobank-prosjektet UUS 2001, 2002, 2003, 2004:	**PE**	**C**	**DM: DM1, DM2, GDM-kost, GDM-insulin**		**DM+PE**
Mors alder i år i dag				**Siste menses dato:**	
Graviditeter siden sist?				Nå: dag i menssyklus (dag 1 er første mensdag, normalt 28 d syklus)	
Fødsler siden sist?				**Prevensjonsbruk (hva):** p-pille, IUD (kobber eller hormonspiral= Levonova/Mirena), kondom, sterilisert (mannen eller damen)t	J/N
Totalt (i dag): Gravida (Antall graviditeter)				**Gravid nå?**	J/N
Totalt (i dag): Para (antall fødsler etter uke 16)				**Siste graviditet/fødsel, hvor mange mnd siden siste gang?**	
				Ammer? (mnd siden siste partus?)	J/N
Andre sykdommer:	J/N	Hvilke sykdommer:			
Sykdom etter sectio-biobank-deltakelse (i 200_____)?	J/N	Hvilke sykdommer:		**Antall ganger preeklampsi**	
Medikamentbruk nå?	J / N	Hvilke medikamenter?			
				HVIS DIABETES noen gang:	
Røyker nå	J / N	stk/dag:		**DM før/ under/ etter svangerskap?**	
Snus nå	J / N	stk/dag:		**Dia type I (antall år)**	J / N
				Dia type II (antall år)	J / N
Mors høyde (cm)				**Har hatt GDM i et svangerskap? (antall ganger?)**	J / N
Mors vekt (kg), t-skjorte og truse på				sist HbA1C (Dato:)	
Mors livvidde, smalest (cm)				Nå: Kostregulert diabetes bare?	J / N
Mors livvidde, mellom nederste ribbe og hoftekam (cm)				Nå: perorale antidiabetika?	J / N
Mors hoftevidde (hoftekamknutenivå) (cm)				Nå: insulinregulert diabetes?	J / N
				Insulinmengde pr døgn:	
Mors BT i dag, hvert mål nærmeste 2 mmHG (3 målinger etter 5 min hvile, sittende, bruk høyre arm, de 2 siste målinger teller og brukes til å regne et matematisk gj.snitt) Mansjettbredde ca 1/3 av overarmvidden.	**Syst**	**Diast**		hurtigvirkende (IE)	
BT1				langsomtvirkende (IE)	
BT1				Total insulinbruk (IE)	
BT3					
URIN STIX MOR; (skriv alltid protein: 0, +1, +2, eller +3. anfør ellers bare om andre variabler slår ut på stix)			Hvis + urin, send prøve til mikrobiol us, UUS, fyll ut rekv skjema med Annetine Staffs klistremerke. Skriv på skjema at urin stix positiv på … Kvinnen frisk, utelukke Urinveisinfeksjon. Ingen antibiotikabruk pågående. Gi Annetine beskjed, Annetine følger opp prøven og gir svar til mor.		
Antall rør med urin i fryser (x rør):					
Antall blodprøver i fryser mor:	0.2 ml	0.5 ml	1 ml		
serum					
edta-plasma					
citrat-plasma					

Appendix 2

edta-cellerest	J/N				
Paxgene rør, antall nunc?					
Heparinrør til Glutathion					
BARNETS KLISTREMERKE			Barnets Navn og fødselsnummer		
CHASE-nummer: CB1-149					
Barnets kjønn	M/F				
Barnets alder i år i dag				Barnet går i barnehage/ dagmamma/ skole?	
Barnets alder i mnd i dag					
Barnets vekt i dag (kg)					
Barnets høyde i dag (cm)					
Barnets smaleste livvidde i dag (cm)					
Barnets livvidde i dag(målt midt mellom nederste ribbe og hoftekam)(cm)					
Barnets hoftevidde (hoftekam) i dag (cm)					
	SYST	**DIAST**			
Barnets BT i dag (3 målinger etter 5 min hvile, sittende, bruk høyre arm, de 2 siste målinger teller). Mansjettbredde ca 1/3 av overarmvidden			*Hvis + urin, send til micribiol us med Heidi Ramstads rekvmerke og gi henne beskjed, hun følger opp pasienten.*	*Hvis + urin, send til micribiol us med Heidi Ramstads rekvmerke og gi henne beskjed, hun følger opp pasienten.*	
BT1			*Gi ved positiv urin beskjed til barnets mor om at urinen sendes til us og at hun får svar fra barnelegen uansett fun*		
BT2					
BT3					
URIN STIX BARN (skriv alltid protein: 0, +1, +2, eller +3. anfør ellers bare om andre variabler slår ut på stix)			Hvis + urin, send prøve til mikrobiol us, UUS, fyll ut rekv skjema med Heid Ramstads klistremerke. Skriv på skjema at urin stix positiv på … Barnet frisk, utelukke Urinveisinfeksjon. Ingen antibiotikabruk pågående. Gi Heidi beskjed, Heidi følger opp prøven og gir svar til mor.		
Antall rør med urin i fryser (x rør):			Gi ved positiv urin beskjed til barnets mor om at urinen sendes til us og at hun får svar fra barneleg		
Barnets medikamentbruk i dag					
Frisk etter fødsel?	**JA**				
	NEI-spesifiser				
Innhentet barnets vektpercentilskjema fra mor, evt hun henter fra helsestasjon?					
	vekt	**høyde**	**VektHøydePercentil**	**HøydeAlderPercentil**	
Fødsel					
6 mnd					
12 mnd					
4 år					
Blodprøver i fryser barnet:	0.2 ml	0.5 ml	1 ml		
serum					
edta-plasma					
citrat-plasma					
edta-cellerest	J/N				

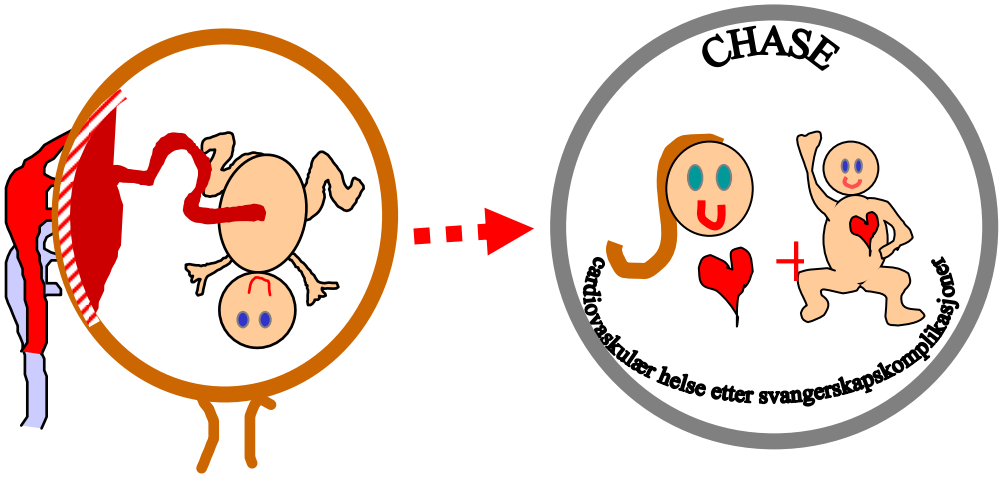
19.08.08

Kort spørreskjema om kosthold og fysisk aktivitet for kvinnen (mor) som er med i CHASE-studien

Ta med ferdig utfylt skjema på avtalt undersøkelse ved Ullevål universitetssykehus

Skjemaet er fylt ut av: _____

Dato: _____



Skjemaet består av 3 sider
og inneholder
11 spørsmål

19.08.08

KOSTHOLD

Vi spør om dine spisevaner slik de vanligvis er. Vi er klar over at kostholdet varierer fra dag til dag.

Prøv derfor så godt du kan å gi et "gjennomsnitt" av dine spisevaner.

Ha det siste året i tankene når du svarer. Der du er usikker, anslå svaret.

1. Hvor mye drikker du vanligvis av følgende drikker?

Sett ett kryss for hver drikke. (½ liter = 3 glass)

	Drikker Aldri/sjelden	1-3 glass pr mnd	1-3 glass pr uke	4-6 glass pr uke	1-3 glass pr dag	4-6 glass pr dag	7 glass el mer pr dag
Helmelk (søt og sur), glass	☐	☐	☐	☐	☐	☐	☐
Lettmelk (søt og sur), glass	☐	☐	☐	☐	☐	☐	☐
Skummet melk, ekstra lett lettmelk (søt og sur), glass	☐	☐	☐	☐	☐	☐	☐
Juice, most, glass	☐	☐	☐	☐	☐	☐	☐
Sukkerholdig leskedrikk (brus, saft), glass	☐	☐	☐	☐	☐	☐	☐
Kunstig søtet leskedrikk (lett brus, saft), glass	☐	☐	☐	☐	☐	☐	☐
Øl, glass	☐	☐	☐	☐	☐	☐	☐
Vin, glass	☐	☐	☐	☐	☐	☐	☐

2. Hva pleier du å smøre på brødet? (sett kun ett kryss)

Bruker ikke smør/margarin	Meierismør	Bremykt	Brelett	Melange, Per, Soya margarin i pakke	Soyamargarin i beger (eks Soft Flora)	Lettmargarin (eks Soft Light)
☐	☐	☐	☐	☐	☐	☐

3. Hvis du bruker smør eller margarin på brødet, hvor mye pleier du å smøre på per skive?

(sett kun ett kryss)

En porsjonspakning på ca 12 g rekker til omtrent	1	2	3	4	5	skiver
	☐	☐	☐	☐	☐	

19.08.08

4. Hvor ofte bruker du vanligvis? (sett ett kryss for hvert kosttilskudd)

	Aldri/ Sjelden	1-3 ganger Pr mnd	1-3 ganger pr uke	4-6 ganger pr uke	1 gang pr dag	2 ganger pr dag	3 ganger pr dag	4 ganger eller flere pr dag
Tran	☐	☐	☐	☐	☐	☐	☐	☐
Vitamintilskudd	☐	☐	☐	☐	☐	☐	☐	☐

5. Hvor ofte spiser du vanligvis de følgende matvarer i løpet av en uke? (Sett ett kryss for hver matvare)

	Aldri/ Sjelden	1-3 ganger Pr mnd	1-3 ganger pr uke	4-6 ganger pr uke	1 gang pr dag	2 ganger pr dag	3 ganger pr dag	4 ganger eller flere pr dag
Loff/ fint rundstykke, skiver	☐	☐	☐	☐	☐	☐	☐	☐
Mellomgrovt brød/grovt rundstykke, skiver	☐	☐	☐	☐	☐	☐	☐	☐
Grovbrød, skiver	☐	☐	☐	☐	☐	☐	☐	☐
Ost som pålegg og i retter, skiver	☐	☐	☐	☐	☐	☐	☐	☐
Poteter i en eller annen form f.eks kokt potet, potetmos, stekt potet, bakt potet	☐	☐	☐	☐	☐	☐	☐	☐
Grønnsaker, for eksempel rå grønnsaker, salater, kokte grønnsaker, stekte grønnsaker, grønnsaksretter, grønnsaksjuice	☐	☐	☐	☐	☐	☐	☐	☐
Frukt og bær, alle typer som f.eks frisk frukt/bær, kompott, hermetikk, juice osv.	☐	☐	☐	☐	☐	☐	☐	☐
Fisk til middag	☐	☐	☐	☐	☐	☐	☐	☐
Kjøtt til middag	☐	☐	☐	☐	☐	☐	☐	☐

Måltider

6. Hvor ofte pleier du å spise følgende måltider i løpet av en uke? (sett ett kryss for hvert måltid)

	Aldri/ Sjelden	1 gang i uken	2 ganger i uken	3 ganger i uken	4 ganger i uken	5 ganger i uken	6 ganger i uken	Hver dag
Frokost	☐	☐	☐	☐	☐	☐	☐	☐
Formiddagsmat/lunsj	☐	☐	☐	☐	☐	☐	☐	☐
Middag	☐	☐	☐	☐	☐	☐	☐	☐
Kveldsmat	☐	☐	☐	☐	☐	☐	☐	☐

19.08.08

7. Hvor mange ganger pleier du å spise ett eller annet utenom hovedmåltidene (f eks godteri, frukt, brødskive) i løpet av dagen?

Sjelden	1 gang om dagen	2 ganger om dagen	3 ganger om dagen	4 ganger om dagen	mer enn 4 ganger om dagen
☐	☐	☐	☐	☐	☐

Fysisk aktivitet

8. Hvor mange av de siste 7 dager har du gått eller syklet til eller fra jobb, butikk eller lignende i moderat tempo i totalt minst 30 minutter? (Bolker på 10 minutters varighet teller, dvs at 3x10 minutter fører til 30 minutters aktivitet.)

Ingen dager	1 dag	2 dager	3 dager	4 dager	5 dager	6 dager	Hver dag
☐	☐	☐	☐	☐	☐	☐	☐

9. Hvor mange av de siste 7 dager har du utført fysisk aktivitet i fritiden som har medført moderat høy puls i totalt minst 30 minutter? (Bolker på 10 minutters varighet teller, dvs at 3x 10 minutter fører til 30 minutters aktivitet).

Ingen dager	1 dag	2 dager	3 dager	4 dager	5 dager	6 dager	Hver dag
☐	☐	☐	☐	☐	☐	☐	☐

10. Hvor mange av de siste 7 dager har du utført fysisk aktivitet i fritiden som har medført høy puls i minst 20 minutter (sammenhengende)?

Ingen dager	1 dag	2 dager	3 dager	4 dager	5 dager	6 dager	Hver dag
☐	☐	☐	☐	☐	☐	☐	☐

11. Var de siste 7 dagene representative for det vanlige fysiske aktivitetsnivået ditt?

Ja	Nei, var mer fysisk aktiv enn vanlig	Nei, var mindre fysisk aktiv enn vanlig
☐	☐	☐

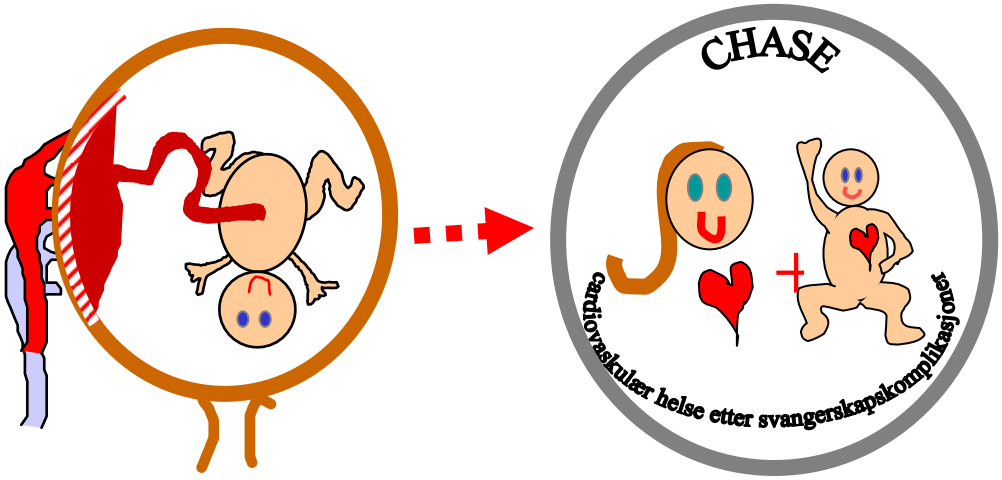
19.08.08

Kort spørreskjema om kosthold og fysisk aktivitet for barnet som er med i CHASE-studien

Skjemaet fylles ut av mor og tas med på avtalt undersøkelse ved Ullevål universitetssykehus

Skjemaet er fylt ut av: _____

Dato: _____



Skjemaet består av 3 sider
og inneholder
8 spørsmål

KOSTHOLD

I det følgende spør vi om barnets spisevaner slik de vanligvis er. Vi er klar over at kostholdet varierer fra dag til dag. Prøv derfor så godt du kan å gi et "gjennomsnitt" av barnets spisevaner.

Ha det siste året i tankene når du svarer. Der du er usikker, anslå svaret.

1. Hvor ofte pleier barnet å spise følgende måltider i løpet av en uke? (sett ett kryss for hvert måltid)

	Aldri/ Sjelden	1 gang i uken	2 ganger i uken	3 ganger i uken	4 ganger i uken	5 ganger i uken	6 ganger i uken	Hver dag
Frokost	☐	☐	☐	☐	☐	☐	☐	☐
Formiddagsmat/lunsj	☐	☐	☐	☐	☐	☐	☐	☐
Middag	☐	☐	☐	☐	☐	☐	☐	☐
Kveldsmat	☐	☐	☐	☐	☐	☐	☐	☐

2. Hvor mye drikker barnet vanligvis av følgende drikker? (sett ett kryss for hver drikke)
(3 glass = ½ liter)

	Drikker Aldri/sjelden	1-3 glass pr mnd	1-3 glass pr uke	4-6 glass pr uke	1-3 glass pr dag	4-6 glass pr dag	7 glass el mer pr dag
Helmelk (søt/sur)	☐	☐	☐	☐	☐	☐	☐
Lettmelk (søt/sur)	☐	☐	☐	☐	☐	☐	☐
Ekstra lett lettmelk	☐	☐	☐	☐	☐	☐	☐
Skummet melk (søt/sur)	☐	☐	☐	☐	☐	☐	☐
Appelsinjuice	☐	☐	☐	☐	☐	☐	☐
Saft med sukker	☐	☐	☐	☐	☐	☐	☐
Saft kunstig søtet	☐	☐	☐	☐	☐	☐	☐
Brus med sukker	☐	☐	☐	☐	☐	☐	☐
Lett brus, kunstig søtet	☐	☐	☐	☐	☐	☐	☐

3. Bruker barnet vanligvis smør/margarin på brødskiven? Ja ☐ Nei ☐

4. Hvor mange ganger spiser barnet følgende matvarer? (sett ett kryss for hver matvare).

	Aldri/ Sjelden	1-3 ganger pr mnd	1-3 ganger pr uke	4-6 ganger pr uke	1 gang pr dag	2 ganger pr dag	3 ganger pr dag	4 ganger eller flere pr dag
Kokte poteter	☐	☐	☐	☐	☐	☐	☐	☐
Pommes frites	☐	☐	☐	☐	☐	☐	☐	☐
Grønnsaker	☐	☐	☐	☐	☐	☐	☐	☐
Frukt, bær	☐	☐	☐	☐	☐	☐	☐	☐
Grovbrød	☐	☐	☐	☐	☐	☐	☐	☐
Fisk til middag	☐	☐	☐	☐	☐	☐	☐	☐
Pizza	☐	☐	☐	☐	☐	☐	☐	☐
Hamburger/pølse med brød/ kebab	☐	☐	☐	☐	☐	☐	☐	☐
Godterier	☐	☐	☐	☐	☐	☐	☐	☐
Sjokolade	☐	☐	☐	☐	☐	☐	☐	☐
Potetgull og lignende	☐	☐	☐	☐	☐	☐	☐	☐
Peanøtter	☐	☐	☐	☐	☐	☐	☐	☐
Tran, trankapsler	☐	☐	☐	☐	☐	☐	☐	☐
Vitamintilskudd	☐	☐	☐	☐	☐	☐	☐	☐

FYSISK AKTIVITET

5. Utenom skoletid/barnehagen: <u>Hvor ofte</u> driver barnet idrett eller mosjonerer så mye at barnet blir andpusten og/eller svett? (sett ett kryss)

- Aldri ☐
- Mindre enn en gang i mnd. ☐
- 1-3 ganger i mnd. ☐
- 1 gang i uka ☐
- 2-3 ganger i uka ☐
- 4-6 ganger i uka ☐
- Hver dag ☐

6. Utenom skoletid/barnehage: <u>Hvor mange timer i uka</u> driver barnet idrett eller mosjonerer så mye at barnet blir andpusten og/eller svett? (sett ett kryss)

Ingen	☐
Omtrent ½ time	☐
Omtrent 1 time	☐
Omtrent 2-3 timer	☐
Omtrent 4-6 timer	☐
7 timer eller mer	☐

7. Utenom skoletid/barnehage: <u>Hvor mange timer per dag</u> pleier barnet å se på TV og/eller sitte foran PC'en eller bruker andre spillkonsoller (feks Nintendo Wii, Nintendo DS, Playstation, X-box 360)? (sett ett kryss)

Ikke i det hele tatt	☐
Mindre enn en ½ time	☐
½ -1 time	☐
2-3 timer	☐
4 timer	☐
Mer enn 4 timer	☐

8. Hvilken utdanning har barnets mor og far? (sett ett kryss for høyest fullførte utdannelse hos mor og ett kryss for høyest fullførte utdannelse hos far)

	Mor	**Far**
9-årig skole eller kortere	☐	☐
Grunnkurs/ettårig utdannelse utover 9-årig skole	☐	☐
Videregående skole/Gymnas/Yrkesskole (3-årig)	☐	☐
Høyskole- eller universitetsutdanning på 4 år eller mindre	☐	☐
Høyskole- eller universitetsutdanning på mer enn 4 år	☐	☐
Annet	☐	☐

GLUKOSEBELASTNINGER
- 2 h, GL0T og GL2T
- 7 punkts i 2 h (Barn), GLBA
- Forlenget

1. Godkjenning

Dato 29.06.2001	Skrevet av / revidert av: Siri B-W Aagenæs, Kristin Sørlie og Knut Lande Signatur: Kristin Sørlie Knut Lande
Dato 29.06.2001	Seksjonsledere: Siri B-W Aagenæs, Nils Tryland og Knut Lande Signatur:
Dato 29.06.2001	Kvalitetssikringsansvarlig: Gro Siri B. Lillesund Signatur: Gro Siri B. Lillesund

KOPI

2. Endringer

2.1 Viktigste endringer fra forrige versjon: Ny

2.2 Endringer i denne versjon:

Dato	Punkt/ bokstav	Endring	Sign.

3. Distribueres til:

Kvalitetssikringsansvarlig (1), Kvalitetshåndbok Klinisk kjemisk avd. (1), Prøvetaking-seksjonen (13), Integra (1) I alt: 7 stk.

4. Introduksjon

4.1 Kvalifisert personell:

Bioingeniører og helsesekretærer som har fullført prøvetakingsopplæringen og denne SP. Opplæring i tillaging av glukose-oppløsning og prosedyrer rundt praktisk gjennomføring. Bioingeniører/helsesekretærer må ha gjennomført belastningen sammen med kvalifisert personell en gang, før de kan utføre belastningen alene.

4.2 Andre SP det kreves kunnskap om for å utføre belastningen:

SP Venepunksjon
SP Hudpunksjon, kapillærprøve
Brukermanual for B-glukose ved pasientnær analysering (PNA).

4.3 Ansvar: De som utfører belastningen har plikt til å følge denne SP.

Preparering/Tillaging:
Til voksne:
75 g D-glukose løsés opp i 250-300 ml kokende vann.
Tilsett ¼ ts sitronsyremonohydrat granulert og eventuelt isbiter.
Sett parafilm over muggens åpning og sett glukoseløsningen til avkjøling i kjøleskap over natten.
Til barn:
Mengden glukose beregnes pr. kg legemsvekt, maksimalt 75 g. Vannmengden reduseres tilsvarende slik at konsentrasjonen i løsningen blir som for voksne (ref 5.3). Løsningen bestilles på Ullevål apotek dagen i forveien: Oppgi pasientens alder og vekt:

Barnets alder	D-glukose
0-18mnd.:	2,50 g/kg
18 mnd.-3 år:	2,00 g/kg
3-12 år:	1,75 g/kg
12 år-->	1,25 g/kg

Helsefare: Ingen.
Avfallshåndtering: Ingen spesielle prosedyrer.

7.3 Utstyr:
Prøvetakingsutstyr til venepunksjon eller hudpunksjon, kapillærprøve..
Vannkoker, målebeger, mugge, skje, plastkrus, Evt. isbiter.

8. Prøvematerialet
8.1 Pasientforberedelse:
Ved bestilling av glukosebelastning gjøres rekvirenten oppmerksom på at pasienten møter om morgenen, etter 8 timers faste uten kaloriinntak men der drikke av vann er tillatt. Pasienten bør ikke røyke i fasteperioden. De siste 3 døgn må pasienten ha inntatt normal kost (minimum 150 g karbohydrater per. døgn), ha utvist normal fysisk aktivitet og ikke vært akutt syk. (Videre at pasienten skal holde seg i ro i 2 timer under belastningen, og ikke røyke). Se 9.1.

Ved bestilling av glukosebelastning på barn:
Notér barnets vekt. Glukoseoppløsningen bestilles fra apoteket dagen i forveien. Se 7.2

8.2 Prøvetaking:
Voksne: Venøs prøvetaking /evt. kapillær prøvetaking.
Barn: Venøs eller kapillær prøvetaking.
Prøvebeholder: Vakuumrør/mikrotainer m/gel.
Nødvendig volum: 250/100 µl.
Materiale: Serum/blod.

8.3 Prøvebehandling
Oppbevaringssted: Før analysering: Romtemperatur/kjøleskap.
Etter analysering: Kjølerom i romnr. 546A
Holdbarhet: 48 timer for serum på gel. 2 timer for serum uten gel.
Helsefare: Biologisk materiale.
Avfallshåndtering: Se egen SP for håndtering av biologisk materiale.

13. a Glukosebelastning 2 h. Kortversjon.

- Glukosebelastning må avtales på forhånd. Avtalen skrives i almanakk for "Belastninger". For barn må også vekt og alder noteres.
- Glukosebelastning 2 h brukes vanligvis på voksne. Avklar med rekvirenten om denne belastningstypen skal brukes på barn, eller om det bør være 7 punkts i 2 timer.

Glukoseløsning til voksne:

- Løs 75 g D-glukose i 250-300 ml kokende vann.
- Tilsett ¼ ts sitronsyremonohydrat granulert og eventuelt isbiter.
- Sett parafilm over muggens åpning og sett glukoseløsningen til avkjøling i kjøleskap over natten.

Glukoseløsning til barn:

- Mengden glukose beregnes pr. kg legemsvekt, maksimalt 75 g. Vannmengden reduseres tilsvarende slik at konsentrasjonen i løsningen blir som for voksne.. Løsningen bestilles på Ullevål apotek dagen i forveien: Oppgi pasientens alder og vekt:

Barnets alder	D-glukose
0-18mnd.:	2,50 g/kg
18 mnd.-3 år:	2,00 g/kg
3-12 år:	1,75 g/kg
12 år-->	1,25 g/kg

Gjennomføring av belastningen:

- På voksne tas prøven på SST-rør og sentrifugeres så snart som mulig og innen 2 timer. For barn og ved vanskelig prøvetaking av voksne kan også strimmel-metode brukes.
- Ta fastende prøve til s-glukose. Oppgi prøvetakingstidspunkt (GL0T).
- Glukoseoppløsningen drikkes i løpet av 5-10 min
- Ta prøve etter 2 timer til s-glukose. Oppgi prøvetakingstidspunkt (GL2T)
- Pasienten bør være i ro og må ikke spise og/eller drikke, røyke før siste prøve er tatt.
- Ved eventuell forlenget glukosebelastning, tas prøve etter 3 timer til s-glukose.

Registrering i labdatasystemet:

- Registrer analysene GL0T for fastende prøve og GL2T, og legg inn klokkeslett for prøvetaking for begge prøvene. Det må være eget prøvenummer for hvert prøvetidspunkt.
- Svar blir utgitt som
  GL0T besvares som: Glukose bel 0 timer
  GL2T " Glukose bel 2 timer.
- Ved forlenget glukosebelastning, registreres denne som vanlig s-glukose. Legg inn klokkeslett for prøvetaking. Kommentaren "glukose etter 3 timer" legges inn.

Kommentarrutiner:

- Laboratoriet vurderer og kommenterer ikke resultatet av belastningen.
- Eventuelle opplysninger som er aktuelle for belastningens kvalitet legges inn som kommentar til prøven.

Avslutning: Flergangsutstyr leveres til vask i 5. etg. Midtblokken.(Vaktrom nr.542)